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FOOD TECHNOLOGY

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FOOD TECHNOLOGY

VOLUME 2 JULY, 1948 NUMBER 3

Editorial

An Opportunity and an Obligation

More frequent references in the news, as well as the tone and temper of lectures, personal remarks, and a livelier interest in books dealing with man's eternal preoccupation with food, seem to presage a more realistic attitude and understanding of food problems by the general public.

An imbalance always exists between any population and its food supply. There has never been a time when the entire population of the world has been adequately fed. Food technology symbolizes and epitomizes man's travail, research, failures, and successes in his struggle for survival. It should be a source of pride for the food technologist to be intimately associated with a thing so fundamental to man and his civilization.

The food technologist is temporarily eclipsed by the nuclear physicist in matters of public recognition and personal identity. Regardless of the ultimate catastrophe, or millenial benefits, which may result from applications of atomic fission, man will continue to depend upon the more readily recognizable forms of food for his subsistence. Regardless of how much his knowledge and technology be extended, his diet will not consist, in any great measure, of isotopes and mesons, as such.

It is essential that the food technologist broaden his comprehension of the social, economic, and political aspects of food. The abortive attempts to change the accepted diets of certain populations, the political maneuvers disguised as humanitarian projects, and the wanton waste of food to support artificial economies, make it obligatory that the food technologist be prepared to critically evaluate collateral issues related to food.

We have become increasingly aware of the maladjustments of sections of the world's populations to the available food supply. Recognizing the controversies inherent in any discussion of this problem, we still feel that clear thinking in this connection is incumbent on each of us.

As food technologists we have a duty and an obligation, not only in the production, processing, and distribution of food, but in a broad basic understanding of its ultimate social, moral, and political aspects.

Obviously, it is not within the scope of an editorial, much less within the ability of the writer, to deal with any single phase of the problem. We wish, however, to direct attention to a publication dealing with certain aspects of the world's food problems, with which we were much impressed. This publication is "Starvation Truths, Half Truths, Untruths," by Frank A. Pearson and Don Paarlberg, New York State College of Agriculture at Cornell University, Ithaca, New York.

The authors address themselves to the economics of the problem, which they characterize as "dismal." They deliberately avoid other aspects fraught with controversy and hysteria, and incidentally conclude that man is no novice regarding the economics of feeding himself.

The fact that they lean heavily on Malthus in no way dates their thinking. Their treatment of the problems of "Adjusters of Population" and "Adjusters of the Food Supply" seems credible in the light of historical fact. A few conclusions regarding cannibalism may startle some readers. However, the authors presumably have

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not attempted to draw interest to themselves, nor their thesis, by dressing old facts in bizarre garb. The article is short (39 pages), with many references for those who might wish to carry their inquiry further.

This is not intended as a review of the article, nor an attempt to promote its distribution. Our present purpose is to express our belief that the article clearly defines certain issues on which it would be well for the food technologist to formulate considered opinions.

Ill considered tampering with the world's food supply, though not as spectacular as "Push Button War," can have consequences just as grave. The food technologist is a member of an organization which is international in its interests; the food technologist's judicious opinions, therefore, must sooner or later be considered in the formulation of international policies.

This is written, not as a challenge to your current opinions, but as a suggestion that you formulate your opinions as a scientist, and as a food technologist, on all matters pertaining to food.

Our future will be determined by the wisdom employed in the formulation of international policies of feeding the world. This is an endeavor, and a venture in which no one could serve more ably than the food technologist.

M. L. Laing.

Editorial

Congratulations, Doctor Woolley

FOOD TECHNOLOGY adds its congratulations to those currently being showered upon Doctor D. W. Woolley, brilliant young biochemist who is an associate member of the Rockefeller Institute. It has been announced recently that the Eli Lilly Award in Biochemistry will be presented to Dr. Woolley at the fall meeting of the American Chemical Society. Winning awards sponsored by the Lilly Company is beginning to be a routine matter to Woolley; in 1940 he received a similar award ($1,000 and a medal) in bacteriology. He is the first to achieve the distinction of both awards. Additional honors have come to him in the form of the Mead Johnson Award of the American Institute of Nutrition, the Research Award of the American Pharmaceutical Manufacturers, and selection as one of the Harvey Lecturers of the New York Academy of Medicine. A product of Canada, Woolley completed his undergraduate training at Alberta in 1935 and followed this with the masters (1936) and doctorate (1938) at the University of Wisconsin where his major was biochemistry. In 1938 a travelling fellowship of the 16th International Physiological Congress took him to England and in 1947 he attended the International Congress of Microbiology in Copenhagen. In 1945 he married Janet McCarter, who holds a Ph.D. in bacteriology from the University of Wisconsin and who was a joint author in an early publication in bacteriology. Dr. Woolley is a member of many scientific societies including the American Institute of Nutrition, the American Chemical Society, the Society of American Bacteriologists, the Society of Biological Chemistry, the New York Academy of Medicine, the New York Academy of Science, and the Society for Experimental Biology and Medicine.

At Wisconsin his interest in biochemistry led him into problems relating to bacterial nutrition and his contributions have done much to justify the inclusion of bacterial physiology in the general area of comparative biochemistry. Especially important are his contributions on the antagonism between certain metabolic compounds and their structural analogs. His recent contributions relate to fundamental problems in the action of the animal and bacterial viruses.

While Dr. Woolley will long be remembered for the importance of his individual research publications, mention should also be made of the fact that he is typical of a group of young (35 years or less) scientists who today are bringing together, with decisive strikes at fundamental problems, many phases of biochemistry, bacteriology, nutrition, and physiology. With many investigators, attack on such problems calls for a cooperating team; with Woolley, as with few others of his age, the team needs but one man—Woolley. Again, our congratulations.

L. S. MCCLUNG.

STUDIES ON *CLOSTRIDIUM BOTULINUM* IN FROZEN PACK VEGETABLES

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AND

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Critical examination is given in the results reported in this paper of the possibility of toxic spoilage of frozen pack vegetables. The authors report the failure of a wide variety of frozen vegetables to become toxic when thawed and held at room temperature for 2 days. The data presented yield no evidence that hermetically sealed containers would introduce a public health hazard if used in the freezing preservation of vegetables.

INTRODUCTION

The question whether frozen foods might give rise to botulism first attracted the attention of bacteriologists and public health workers about 1930. At that time the frozen food industry became interested in the possible application of its methods to the preservation of vegetables. The freezing of fruits and berries was already well established, but had not created apprehension from the public health viewpoint. It was recognized that the natural acidity of these foods is unfavorable for the growth of food-poisoning bacteria, including *Clostridium botulinum*. This is not the case, however, with most vegetables, and it was feared that such items as peas and corn, which may become toxic when imperfectly heat-processed, might also prove dangerous when frozen. It was considered possible that toxin might form during pre-freezing delays and persist during storage, or be produced during distribution, through failure to keep the vegetables frozen. The greatest hazard was believed to be in the home, where the vegetables might be allowed to stand at a temperature favorable to the growth of *C. botulinum*.

In connection with these questions, Tanner and Wallace *(21)* reported that frozen pack vegetables inoculated with detoxified spores of *C. botulinum* became toxic in 3 to 6 days at room temperature. Berry *(1, 2)* did not find toxin in frozen pack peas and snap beans in tin cans and fiber containers that had stood at room temperature from 3 days to 3 weeks. He suggested that, even when the packs had been artificially inoculated with *C. botulinum*, the competition of other bacteria prevented the growth of *C. botulinum*. Straka and James *(18, 19)* reported development of toxin in both inoculated and uninoculated peas. The same authors *(20)* stated that some samples, inoculated as well as uninoculated, developed toxin in 7 days at 50° F. (10° C.). However, Edmondson, Giltner, and Thom *(7)* and Prescott and Geer *(11)* reported that toxin was not formed by *C. botulinum* at 50° F. even after extended incubation. Wallace and Park *(23)* stated that they had found toxin in frozen food inoculated with detoxified spores of *C. botulinum* immediately after defrosting. Remarks on some of these contradictory findings, which gave rise to concern among packers in the early 1930's, are offered in the discussion section of this paper.

The carton, which on theoretical grounds was considered safer by public health officials than the tin can, was adopted as the retail container for frozen foods. Unlike

* Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

the can, it can neither be vacuumized nor hermetically sealed, and was thus believed less likely to permit growth of anaerobic bacteria. Moreover, it is distinctive enough to lessen the chance of confusion by the consumer between frozen foods, which are perishable at ordinary temperatures, and heat-processed foods.

Commercial packs of frozen vegetables in the United States rose from about 1,000,000 pounds in 1930 to 450,000,000 pounds in 1946. In the same period large quantities of vegetables were frozen in cold storage lockers. Despite the fact that cases of improper handling have occurred in this great quantity of frozen food, no outbreak of botulism has been traced to it. The general opinion is that frozen foods are not a hazard to public health when packed in the usual types of nonhermetic packages.

Recently, however, attention has been focused on the suitability of the tin can as a container for frozen vegetables. Some packers, impressed with its ease of handling and protective qualities, definitely want to use it. They have been restrained from doing so by regulatory authorities, pending specific information on the influence of hermetic sealing and vacuumization of vegetable packs on the growth of *C. botulinum*.

This investigation was undertaken primarily to collect evidence on the type of spoilage occuring in vegetables frozen in tin cans and thawed. Nonhermetic containers, which under *experimental* conditions have been reported to permit the development of toxin, were included in the pack. Also, opportunity was taken to examine a variety of commercially frozen vegetables for the presence of *C. botulinum*, and possible toxin development on standing at room temperature.

Since it has been argued that spoiled foods could be more readily detected from the outward appearance of cartons than of cans, observations were made of the condition of every container after the contents had been allowed to spoil, and before opening.

This report represents the joint contribution of the two cooperating agencies. The experimental inoculated packs of peas, asparagus, and lima beans were prepared in common. Analyses of material were made independently as to time and place, but conformed to a general scheme previously agreed upon. Though there were slight differences in technique, such as in the method of inoculating test animals, no contradictory findings between the two groups occurred.

METHODS AND MATERIALS

Experimental packs, partly inoculated with Clostridium botulinum.

Peas. Freshly harvested peas in pods were held overnight at 33° F. (0.5° C.), shelled by machine, washed, blanched for 90 seconds in steam, cooled in an air blast, and packed in 211 x 400 cans and 12-ounce cartons. These operations were performed in a pilot plant. One-half of the containers for each packing condition was inoculated with 2 ml. of a mixed suspension of *C. botulinum*,[b] containing 5000 spores of type A strain 62 and 5000 spores of type B strain 213. The inoculum was introduced into each can by syringe through a small hole in the lid, and was distributed as evenly as possible over the surface of the peas in each carton. The cans were sealed in air, in nitrogen, and in a vacuum. In nitrogen packing a vacuum desiccator and special soldering technique were used. The vacuum-packed cans, after the pressure had been reduced to approximately 15 mm. Hg, were sealed by a modification of the method of Bryce and Tessier *(4)*. There were 8 variations of pack (see Table 1). After permanent closure all containers were well shaken to distribute the inoculum, and were immediately spread out at a temperature of

[b] Supplied by University of California Laboratory for Research in the Canning Industries.

TABLE 1

Packing Data and Results of Examination of Experimental Inoculated Pack

Type of pack	Containers No. and type	Artificially inoculated	No. examined for toxin	No. toxic	No. cultured for spores	No. cultures toxic after heating in enrichment medium and incubating 10 days
			PEAS			
Air	111 cans	No	41	None	31	None
Nitrogen	100 cans	No	41	None	31	None
Vacuum	110 cans	No	41	None	31	None
Air	150 cans	Yes	41	None	31	29
Nitrogen	125 cans	Yes	41	None	31	29
Vacuum	126 cans	Yes	41	None	31	29
Air	125 cartons	No	41	None	31	None
Air	125 cartons	Yes	41	None	31	30
			ASPARAGUS			
Air	42 cans	No	17	None	14	None
Nitrogen	42 cans	No	17	None	14	None
Vacuum	42 cans	No	17	None	14	None
Air	42 cans	Yes	17	None	14	1
Nitrogen	42 cans	Yes	17	None	14	None
Vacuum	42 cans	Yes	17	None	14	None
Air	63 cartons	No	17	None	14	None
Air	63 cartons	Yes	17	None	14	1
			GREEN LIMA BEANS			
Air	59 cans	No	25	None	19	None
Nitrogen	60 cans	No	25	None	19	None
Vacuum	60 cans	No	25	None	19	None
Air	60 cans	Yes	25	None	19	15
Nitrogen	60 cans	Yes	25	None	19	17
Vacuum	60 cans	Yes	25	None	19	19
Air	66 cartons	No	25	None	19	None
Air	60 cartons	Yes	25	None	19	19

— 10° F. (— 23.3° C.) for 24 hours to ensure rapid freezing. The containers were stored at 0° F. (— 17.7° C.).

Asparagus. In the pilot plant asparagus was cut into pieces about ¾ inch in length to facilitate handling, and blanched for 2 minutes in steam. Treatment thereafter was the same as that described for peas. The pack was accidentally put in a room at 33° F. (0.6° C.) for 3 days, but since there were no signs of spoilage, it was put to freeze at — 10° F. Four containers taken at random yielded aerobic counts on nutrient glucose agar of only 12,000 to 120,000 organisms per gram, and there seemed to be no reason why the pack should not be used.

Lima Beans. Green lima beans were taken from the sorting belt in a freezing plant. They had been blanched in water at 210° F. (99° C.) for 4 minutes. The beans were iced, and stored at 32° F. (0° C.) overnight. The pack was prepared in the manner described for peas.

Packs not artificially inoculated.

Packs of pumpkin and Brussels sprouts were prepared under commercial conditions. The purpose was to acquire information as to whether the vacuumized can would favor the development of *C. botulinum* at 70° F. Although pumpkin is not a favorable medium for the growth of *C. botulinum*, special interest attaches to the pack, which was essentially a pre-cooked frozen food.

Pumpkin. Pumpkin was washed, cut, heated for 25 minutes in steam, put through a finisher, preheated, and held at 160° to 170° F. (71-77° C., approx.) at the filler. Containers were 401 x 411 cans and 1-pound cartons. The cans were sealed in a 15-inch vacuum. The process required about 2 hours from the start until the pack was placed in cold storage at 32° F. It was held at this temperature for 4 hours and then transferred to a room at — 30° F. (— 34° C.) for 24 hours and stored at 0° F.

Brussels Sprouts. The sprouts were sprayed, washed, blanched in steam for 4 minutes, cooled in an ice-water spray, and packed in 10-ounce cartons and 401 x 411 cans. The cans were sealed in a 19-inch vacuum. The containers were held for 1½ hours at 32° F., frozen at — 30° F., and stored at 0° F.

Miscellaneous Commercial Frozen Vegetables. Samples of asparagus, beets, broccoli, Brussels sprouts, carrots, cauliflower, corn, lima beans, peas, peas and carrots, spinach, string beans, wax beans, and yellow squash, were bought in the open market at various times from Jan. 1, 1946 to Jan. 27, 1947, and were tested immediately. An effort was made to obtain vegetables packed in as many different areas as possible. The samples, 303 in all, were in 12-ounce cartons, with the exception of a few experimental units of asparagus, lima beans, and spinach, which were in 307 x 409 cans.

Laboratory Technique. Examinations of experimental lots were made at intervals over a period of 2 weeks to 1 year after packing. The examination consisted of two parts: (1) allowing the food to spoil at 70° F. (21° C., approx.) for 48 to 52 hours and testing for toxin and (2) culturing the freshly thawed food from a duplicate container to determine whether viable spores of *C. botulinum* were present. In culturing, 60 grams of the vegetable was added aseptically to 120 ml. of corn steep liquor-casein medium.[e] The culture was heated at 158° F. (70° C.) for 1 hour, or at 212° F. (100° C.) for 10 minutes, to destroy vegetative cells. It was then either stratified with sterile refined petrolatum or placed in a desiccator chamber (without desiccant) in which the pressure was reduced to water-vapor pressure. Incubation was for 10 days at 86° F. (30° C.) or at 72-77° F. (22-25° C., approx.). Part of the time a similar but unheated culture was incubated in addition. Following incubation, the gross characteristics of the cultures were noted, smears were made, the pH was determined, and clear sterile filtrates were prepared in centrifugal filters, for animal injection.

In testing spoiled food for toxin, 20 ml. of sterile water or saline was added to the contents of the container and, after thorough mixing, the liquid was drained off, slides were prepared for microscopic examination, the pH was taken, and a sterile filtrate for animal injection was prepared.

Mice were used in testing filtrates for toxin. Some comparative evaluations of the intravenous, subcutaneous, and intraperitoneal methods of injection were made. The intravenous injection in the lateral tail vein was easily accomplished after holding the mice in a 95°-98° F. (35°-37° C., approx.) incubator for a few minutes and moistening the tail with xylene. When this method was used the pH of the material was adjusted to 7.2 ± 0.2. The subcutaneous injection was made on the back of the animal near the base of the tail. The time of death varied considerably,

[e] Medium for the cultivation of *C. botulinum:* To tap water adjusted to pH 11, add 0.3% technical casein. This must be ground in; otherwise, lumps form. The best method is to sift casein slowly into the water by grinding. Add 1% corn steep liquor and adjust the pH to 7.2. Finally, add 0.5% commercial dextrose and autoclave. The preparation of the corn steep liquor is as follows: Add 55 parts of corn steep liquor (raw) to 4 parts water. Adjust pH to 8.5, using 40% sodium hydroxide. Heat to 65° C. for one-half hour. Add 1 handful of filter aid per 6 liters. Filter while hot on a Büchner funnel into suction flask. Use filtrate for medium. It should contain 40-52% total solids. Keep in refrigerator.

depending on the method of injection. In a typical experiment using 0.05 ml. filtrate from a toxic culture the time of death after intravenous injection was 1 hour and 50 minutes; after intraperitoneal injection, 5 hours and 5 minutes; and after subcutaneous injection, 18 hours. In routine examination of samples, 0.1 ml. was given when the intravenous method was employed and 0.2 and 0.25 ml. by the subcutaneous and intraperitoneal methods, respectively. All three methods were found reliable and have been employed in these experiments for the determination of the presence of botulinum toxin. Even with the less sensitive subcutaneous method, toxic filtrates diluted 100 times killed mice within 48 hours. The three methods, however, are not equally suitable for all vegetables. Spinach was found to contain a heat-stable substance which killed mice in 39 to 52 hours when injected intraperitoneally, but had no ill effect when injected subcutaneously. Filtrates from green lima beans, when administered intravenously, sometimes produced severe shock instantaneously, but had no such effect subcutaneously. About one-half of the samples reported was tested intraperitoneally, while the remainder was examined by the other two methods. It may be of further interest to note that feeding mice material containing either type A (62) or B (213) toxin in 0.45-0.75 ml. amounts failed to affect the animals, although subcutaneous injections of 0.002 ml. proved fatal.

The animals were observed for 7 days after injection and then discarded. If a mouse died after receiving a given filtrate, a control mouse was given the same filtrate, which had been heated 5 minutes in flowing steam. Death of a mouse receiving the unheated filtrate and survival of the control were considered proof of the presence of toxin. In all cases where culture filtrates from inoculated vegetables proved to be toxic, smears of the cultures showed spores typical of *C. botulinum*. In the few cases where cultures derived from uninoculated vegetables were fatal to mice, confirmation was sought by feeding a guinea pig 2 ml. of filtrate by means of a plastic stomach tube. If no symptoms were observed in 7 days, the filtrate was considered nontoxic. When a filtrate proved toxic on feeding, an autopsy was performed and an antitoxin test made to determine the type of toxin. Two-tenths ml. of types A, B, and polyvalent antitoxin was inoculated intraperitoneally into each of three mice. One-half hour after inoculation, 0.2 ml. of the filtrate was injected intraperitoneally into each mouse. The type of *C. botulinum* producing the toxin was determined by noting which antitoxin gave protection.

RESULTS

Peas. Three hundred and twenty-eight containers of peas, 246 of which were cans, were examined for toxin after standing for 2 days at room temperature. Half of the number had been inoculated. They had been in freezing storage for 2 weeks to 1 year. Irrespective of type of pack, the peas were badly spoiled, as was evidenced by disappearance of green color, sour odor, and the highly turbid liquid drained from the containers. The air and nitrogen packs were "hard swells," while the vacuum-packed cans usually showed slight pressure. The pH of the liquid was, as a rule, between 4.3 and 4.6, the extremes being 4.0 and 5.16. Smears were all much alike, showing principally small Gram-positive rods. Of the 328 samples of spoiled peas tested, none showed evidence of botulinum toxin. It was found that, of 64 unheated samples of freshly thawed peas representing all variations of pack, none developed toxin when incubated anaerobically for 10 days in enrichment medium. In these fermentations the final pH was 4.1 to 4.7. However, when the cultures were heated as previously described before incubation, those containing peas that had been inoculated in almost every case became toxic. The pH of toxic cultures remained above 5.8.

Asparagus. One hundred and thirty-six containers of asparagus, which were representative of the pack, in storage up to 10 months, gave negative tests for toxin after standing for 2 days at room temperature. The asparagus invariably showed much spoilage, having a sour odor, soft texture, and brown discoloration. The air- and nitrogen-packed cans were more or less swollen. Gram-positive coccoid to medium-sized rod-shaped bacteria predominated in the smears. Liquid drained from the spoiled asparagus varied from pH 3.5 to pH 4.2. Attempts to produce toxin from the pack by heating and incubating the material in corn steep-casein medium were successful in only 2 cases, both of which involved artificially inoculated material. As a rule, the pH of the heated asparagus cultures was 4.50 to 5.25 but the bacteria were not morphologically characteristic of *C. botulinum*. Asparagus may not be a good medium for *C. botulinum*, or possibly other bacteria surviving the heating were antagonistic.

Lima Beans. Of 200 containers of the lima bean pack tested over a 7-month period, 50 were cartons and 150 were cans. None was toxic, though without exception the beans were markedly spoiled after standing for 2 days at room temperature. Even the vacuum-packed cans at times showed pressure. pH readings on the drained liquor ranged from 4.50 to 4.95. Irrespective of type of pack, direct microscopical examination showed almost exclusively Gram-positive small rods or coccoids. All enriched cultures prepared from freshly thawed beans, without heating, became quite acid—pH 4.10 to pH 4.38—and none was toxic. Heated cultures of inoculated beans, with few exceptions, were toxic. The pH of most of the toxic cultures remained above 6.0. Uninoculated beans in heated cultures did not develop toxin. Pertinent data pertaining to the results of examination of packs of peas, asparagus, and lima beans are shown in Table 1.

Pumpkin. Twenty-seven cartons and 19 cans of the frozen pumpkin pack were examined over a 10-month period. Because spoilage occurred slowly at 70° F. (21° C., approx.) samples were held 4 days before testing. At the end of that time the pumpkin was slightly sour. There was no toxicity and apparently no effect of the type of container on the kind of bacterial development. Smears showed coccoids and small Gram-positive rods, with an occasional yeast-like form. The pH varied from 5.48 to 5.62. The cultures were not toxic.

Brussels Sprouts. Samples of Brussels sprouts showed brown discoloration and developed a sour odor in 2 days at 70° F. (21° C., approx.) The pH varied from 4.04 to 4.70. The spoiled material from cans and cartons alike contained round or oval Gram-positive bacteria, and was not toxic. Cultures also proved negative for toxin. Twenty-five cartons and 23 cans of this pack were tested in the course of 10 months.

Commercial Packs. In addition to the above experimental packs, 303 commercial samples obtained from retail stores or packers were tested for toxin after spoilage. None was toxic. Of 6 cultures of *C. botulinum* isolated from these samples by the use of enrichment medium and heating, 2 were from spinach in cartons, 2 from spinach in cans, and 2 from lima beans in cartons. All proved to be Type A. Data on commercially packed frozen vegetables are presented for convenience in Table 2.

Appearance of Containers After Spoilage of Contents

Most of the cans sealed in air and nitrogen and a few of those vacuum packed were swollen at the end of the incubation period. The cartons showed little or no external evidence of the spoiled contents. There was no leakage, but in a few cases there was slight bulging. The observations indicate that spoilage of food packed in hermetically sealed cans is more readily detectable from the outward appearance of the containers than spoilage of food packed in cartons.

TABLE 2

Results of Examination of Commercially Frozen Vegetables

Product	No. of cartons	No. of cans	pH after 2 days' incubation at 70° F. (21° C. approx.)	No. toxic after 2 days' incubation at 70° F. (21° C. approx.)	pH after heating in enrichment medium and incubating 10 days	No. cultures toxic after heating in enrichment medium and incubating 10 days
Asparagus	12	3	4.16-5.04	None	5.90-6.10	None
Beets	4		4.14-4.20	None	5.14-5.24	None
Broccoli	26		4.12-4.92	None	4.86-5.02	None
Brussels sprouts	16		4.52-4.64	None	5.08-5.18	None
Carrots	2		3.76	None	4.28-4.54	None
Cauliflower	21		4.06-4.64	None	4.74-4.82	None
Corn	52		4.06-4.60	None	4.72-5.36	None
Baby lima beans	5		4.66-5.42	None	5.66-5.60	None
Lima beans	19	4	4.60-5.42	None	5.50-5.62	2
Mixed vegetables	1		4.36	None	.....	None
Peas	69		4.32-4.98	None	4.30-4.38	None
Peas and carrots	4		4.08-4.14	None	.....	None
Spinach	13	9	4.37-5.96	None	4.92-5.52	4
String beans	31		4.00-4.46	None	4.28-5.48	None
Wax beans	2		3.96	None	4.70-4.82	None
Yellow squash	10		4.02-4.40	None	4.32-4.40	None
Total	287	16				

DISCUSSION

The outstanding result of the work reported here is the failure of a wide variety of vegetables prepared and frozen under commercial or semi-commercial conditions to become toxic when thawed and held at room temperature for 2 days, at which time the samples were badly spoiled. Furthermore, even when 60-gram samples of unheated vegetables were incubated for 10 days anaerobically in an enrichment medium, no toxin developed, nor were clostridia observed microscopically among the organisms in the fermented material. These findings hold without exception for "normal" samples as well as for those artificially inoculated with spores of *C. botulinum* before freezing, and for samples in cans and in cartons alike. However, toxin was demonstrated in a great majority of samples of frozen peas and lima beans which had been inoculated, heated in an enrichment medium, and incubated 10 days anaerobically. Asparagus, under these same conditions, rarely produced toxin. All smears from toxic cultures showed clostridia. It is evident from these experiments that *C. botulinum* could multiply only when most of the organisms naturally present in the vegetables had been killed (Fig. 1).

Many investigators have called attention to the role of specific organisms in hindering the growth of *C. botulinum*, or destroying its toxin. For example, Hall and Peterson *(8)*, Jordan and Dack *(9)*, Dack *(6)*, Sherman, Stark, and Stark *(13, 14)*, Stark, Sherman, and Stark *(15, 16)*, Kayukova and Kremer *(10)*, and Ramon, Richou, and Ramon *(12)* have reported on the antagonistic action of a variety of species, as *Clostridium sporogenes, Escherichia coli, Aerobacter aerogenes, Proteus vulgaris, Streptococcus lactis, Streptococcus thermophilus, Lactobacillus casei*, and *Bacillus subtilis*. Though estimates on numbers of bacteria were not made on the vegetable packs described here except in the case of asparagus, there is no doubt that they harbored many thousand viable organisms per gram as does commercial material even after storage of several months *(17)*. Fermentation, with a marked increase in acidity, quickly ensued when unopened containers stood at room

temperature, pH values with few exceptions falling from above 6.0 to well below 5.0. It would seem that the failure of the unheated inoculated samples to become toxic can be attributed to the rapid development of certain acid-producing bacteria, which Berry *(2)* found to survive a freezing temperature of 15° F. (—9.4° C.) for a long period.

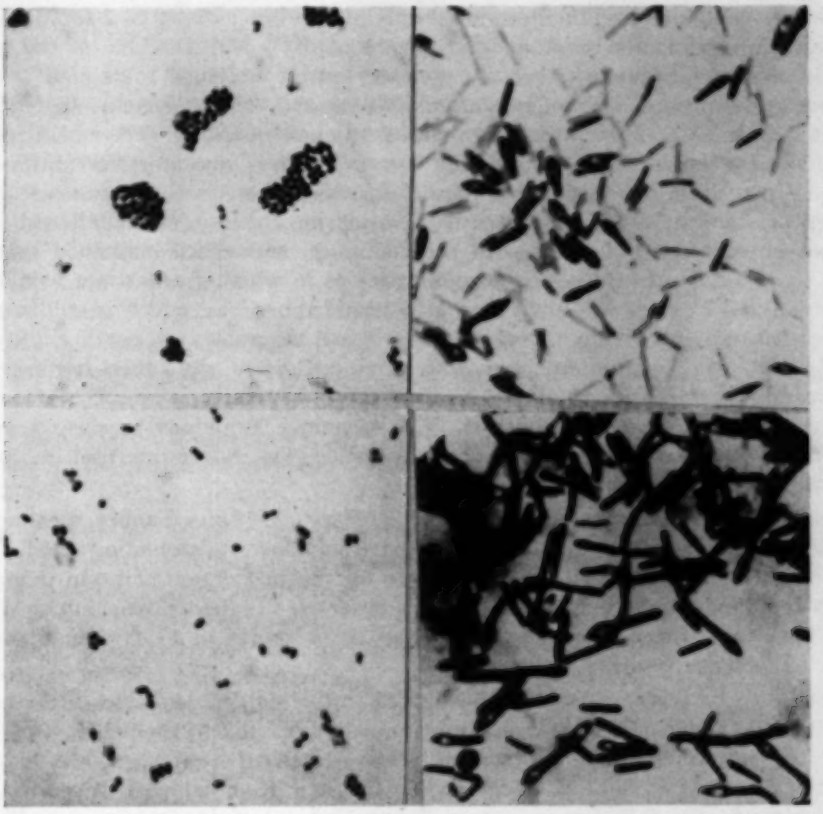

FIG. 1. Photomicrographs showing bacteria in spoiled frozen-pack vegetables artificially inoculated at the time of packing with spores of *Clostridium botulinum.*

Upper left: Frozen-pack peas allowed to spoil at room temperature.
Upper right: Similar peas allowed to spoil after heating to 100° C. (212° F.) 10 minutes.
Lower left: Green lima beans allowed to spoil without heating.
Lower right: Similar green lima beans allowed to spoil after heating.

Vegetables allowed to spoil without heating show no development of *C. botulinum* and were not toxic. The heated samples, those on the right, show development of *C. botulinum;* both proved highly toxic by animal injection.

Commercial frozen pack vegetables, even under the best industrial conditions, contain many thousand bacteria per gram (American Public Health Association, 1946) some of which are acid producers. These appear to be a safeguard against botulism. Results from experimental packs of vegetables, which have been either sterilized and then inoculated with *C. botulinum* spores, or blanched to the point where all but spore formers are destroyed and opportunity for recontamination is not afforded as it is in industrial plants, obviously do not apply to modern commercial frozen pack. Straka and James *(18, 19, 20)* reported 42 toxic samples of peas out

of 390 examined. Of these 42 toxic samples 16 were boiled 10 minutes in a 2.5 percent brine before incubation. Of the remaining 26 toxic samples 21 were severely blanched (4 minutes in boiling water or brine) and packaged in a laboratory presumably without permitting the material to become recontaminated as it is in commercial plants. The 5 remaining toxic samples were not blanched or "brine cooked" but all received an enormous artificial inoculation, 100,000,000 *C. botulinum* spores per container, and then produced only a very low toxicity of 2 to 20 M.L.D., while cultures from the same material produced 3000 M.L.D. One of the factors which must determine whether a vegetable sample becomes toxic and to what extent, is the ratio of viable *C. botulinum* spores and cells to certain other bacteria in the sample. Any of the three treatments mentioned—cooking, severe blanching, or enormous inoculation with botulinum spores—under laboratory conditions will produce a relationship that favors the development of toxin if the material is permitted to stand at room temperature. Considering the samples mentioned above, inoculated with 100,000,000 spores per container, and which contained relatively little toxin (2-20 M.L.D.) the question arises as to whether any toxin would have been detected had this inoculum been a thousand times less, which is still in excess of that naturally occurring in commercially frozen vegetables. A condition favoring the growth of *C. botulinum* was also produced when the other bacteria were destroyed by the brine cooking or the severe blanching without allowing the vegetable to become recontaminated. The technique employed by the workers in question, which made toxin production possible, has no counterpart in modern commercial freezing.

Regarding the reports of Tanner and Wallace *(21)*, and Tanner, Beamer, and Rickher *(22)* it is suggested that the extremely heavy inoculation used by the latter investigators at least was responsible for the production of toxin in some of the frozen foods studied. Nothing has been observed to support the claim of Wallace and Park that frozen foods containing detoxified spores of *C. botulinum* may be toxic as soon as thawed.

Since *C. botulinum* is widely distributed in the soil, its isolation in the present investigation from a few samples of commercially frozen vegetables was to be expected. However, this investigation has shown that frozen vegetables known to contain spores of *C. botulinum* do not become toxic when allowed to spoil at room temperature. The fact that no recorded case of botulism has occurred in 17 years of commercial and locker plant freezing of vegetables is further evidence of the safety of the freezing method. There is nothing in the results obtained in the work described here to indicate that the hermetic container, even when vacuumized, introduces an element of danger when used in the preservation of vegetables by freezing.

Reports on additional vegetables inoculated at the time of packing with spores of *C. botulinum* will be made in due course. Further work is also intended on frozen asparagus which, unlike peas and lima beans, rarely developed toxin even when inoculated and subcultured after heating.

SUMMARY AND CONCLUSIONS

Peas were prepared for freezing under semi-commercial conditions, and packed in tin cans and cartons. One-half of the containers were each inoculated with 5,000 spores Type A and 5,000 spores Type B *Clostridium botulinum*. The pack was stored at 0° F. (— 17.8° C.). Three hundred and twenty-eight representative containers were examined bacteriologically over a 12-month period. None developed

toxin on standing for two days at approximately 70° F. (21° C.). Sixty-four samples, 32 of which had been inoculated, also failed to become toxic when incubated anaerobically for 10 days in enrichment medium. Of 248 samples heated in enrichment medium before incubation, 124 uninoculated samples did not become toxic. Under similar treatment 124 inoculated samples developed toxin in 117 cases (approximately 94%).

Asparagus prepared, stored, and examined similarly to peas did not become toxic when 136 containers, half of them inoculated, were allowed to stand at room temperature for two days. Under enrichment and heating procedures, no toxin developed in 56 normal samples, and only two toxic cultures were derived from 56 containers of inoculated material. Evidently, asparagus is a poor medium for growth of *C. botulinum*.

Lima beans did not develop toxin when 150 cans and 50 cartons, half of which were inoculated, were incubated for two days at room temperature. Seventy-six samples of uninoculated beans tested by heating and enrichment failed to become toxic, but the same number of inoculated samples yielded 70 toxic cultures, or approximately 92%.

Pumpkin frozen in cartons and vacuumized cans did not become toxic when held for four days at room temperature. Twenty-seven cartons and 19 cans were examined. The pack was not artificially inoculated.

Brussels sprouts were not toxic after two days' holding at room temperature. The pack was not artificially inoculated. Twenty-five cartons and 23 cans were tested.

The following commercially frozen commodities, mostly in cartons, were tested for the presence of spores of *C. botulinum* and for toxicity after standing in the unopened containers for two days at room temperature: asparagus, beets, broccoli, Brussels sprouts, carrots, cauliflower, corn, lima beans, baby lima beans, mixed vegetables, peas, peas and carrots, spinach, string beans, wax beans, and yellow squash. In all, 303 samples were tested. None of the spoiled vegetables was toxic. Four cultures of *C. botulinum* Type A were isolated from spinach, and two cultures Type A from lima beans.

In over 1,000 tests all 16 varieties of frozen vegetables except pumpkin became more acid in two days at room temperature, the pH falling in most cases well below 5.0. The vegetables at this time were definitely spoiled. The predominating spoilage organisms, irrespective of whether the vegetables had been inoculated with *C. botulinum* or not, were small Gram-positive rods or coccoids. The microflora appeared to be identical in tin cans and cartons.

In no instance was a spoiled vegetable toxic to test animals.

Spoiled vegetables in cans are more easily detected from outward appearance of the container than are spoiled vegetables in cartons.

The investigation has yielded no evidence that hermetically sealed containers would introduce a public health hazard if used in the freezing preservation of vegetables.

The authors wish to emphasize the fact that the findings in the present research are applicable only to vegetables frozen by current commercial procedures.

ACKNOWLEDGMENT

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A NEW CRYSTALLOGRAPHIC METHOD FOR THE STUDY OF CHANGES IN PROCESSED FOODS[a, b]

(Contribution from the "Reichsforschungsanstalt für Lebensmittelfrischhaltung" in Karlsruhe, Germany)

HANS FRIESS

By the addition of small amounts of biological extracts to cupric chloride solutions, and crystallization under certain definite conditions, it is possible to determine the nature of the biological substances from the characteristic crystallization patterns which result. This method, which was originated by Pfeiffer *(1, 2, 3)*, has been applied to qualitative identification of organic products and to diagnosis of diseases. Heretofore the method has been most useful in medical applications. Thus, Rascher and Trumpp *(4)* reported successful use of the method in 250 cases of cancer. Begouin *(5)* also reported favorable results in cancer diagnosis. Pfeiffer *(6)* confirmed the method for the diagnosis of tuberculosis on a large variety of organic preparation and culture media. The accuracy of the method, in all these cases, lay between 80 and 90 per cent.

Trumpp and Rascher *(7)* also found the method useful for the detection of hormones. By detection of pituitrin in pregnancy urine, they were able to develop an early diagnosis of pregnancy. Morris and Morris *(8)* found that very small amounts of various proteins similarly changed the crystallization figures of cupric chloride, which were formed by polysaccharides. By precipitation of the protein with acetic acid, or acetylation, or saponification, significant changes in the crystallization pattern were brought about. Renewed addition of protein restored the original crystallization figures. Surface active substances such as sulfonates, changed the crystallization pattern in a different manner than proteins. A superposition of its own crystallization figure over that of the polysaccharide resulted.

In these investigations this method was tested as a means of determining changes which are brought about in biological products by heat, freezing, drying, or chemical treatment.

The technique consists essentially in thoroughly mixing 10 cc. of a 5 per cent cupric chloride solution and a definite amount of biological extract and allowing this to crystallize. The amount of added substance used here was varied between 0.1 and 5.0 cc., and in the new method developed, 1 cc. proved to be the most suitable. The extracts are prepared by grinding 20 g. of the particular tissue in a mortar, adding 20 cc. distilled water, allowing it to stand about 15 minutes, and then filtering. The crystallization is carried out on clean, smooth glass plates, about 10 cm. in diameter, bordered with collodion to make flat dishes. These plates are placed in an incubator at 30° C., held at 60 per cent relative humidity, where they will be free from shock.

While the previous applications were based on macroscopic observation of the crystallization figures, in these investigations new characteristic forms were found by microscopic examinations. In the spaces between the large crystals and at their ends, distinct microforms can be seen, which are characteristic of the species, and upon which the changes brought about by added substances may also be observed. In the following examples, only such microforms will be described. They were magnified 45 diameters.

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[a] Translated by Leonora A. Hohl, Division of Food Technology, University of California, Berkeley.

[b] Presented before the Eighth Annual Conference, I. F. T., Phila., Pa. June 9, 1948.

Cupric chloride without additions gives unregulated crystals shown in Fig. 1.

Extracts of raw potato added to cupric chloride give rather broad microcrystalline forms, which branch at the sides and the ends in rather characteristic fashion (Fig. 2). On the other hand, addition of extracts of cooked potato does not yield these forms. These produce thin, long, slightly branched needle-like crystals. The duration of heating seems to play no significant role, one minute of boiling (Fig. 3) yielding practically the same results, as 16 minutes (Fig. 4). The temperature of heating, on the other hand, is critical for the changes in form. In stepwise heating to 50, 60, 70 and 80° C. it is possible to observe a series of transition forms (Figs. 5 to 8). At 50° (Fig. 5) practically no difference can be observed in comparison with the form of the raw potato (Fig. 2). At 60° (Fig. 6), significant changes are already apparent, but in several places it is still possible to see the typical branched forms characteristic of raw potato, only essentially weakened. At 70° (Fig. 7) the forms are quite similar to those of cooked potato, but the needles are still rather wide and blunt. At 80° (Fig. 8) the forms are very similar to those of boiled potato (Fig. 4). The greatest change thus occurs between 60 and 70° C.

Corresponding manifestations are also demonstrable in other products. Raw asparagus juice as biological substance yields longish, slightly branched microcrystal forms (Fig. 9). On the other hand, if cooked asparagus juice is added, these forms do not appear, but only long, thin, unbranched needle-like crystals are found. With asparagus juice, similarly, transition forms of crystals may be observed by stepwise heating between 50° and 80° C., only here the transition zone is narrower. The most striking change in shape also occurs between 60 and 70° C. in this case. Asparagus heated to 80° C. (Fig. 10) yields practically the same forms as cooked asparagus.

Dried peas, on the other hand, upon heating, show practically no change in shape, in comparison with raw dried peas (Fig. 11). Only after 24 hours soaking in water are these changes in shape observable after heating, as Fig. 12 (heated to 80° C.) shows. This observation is in accord with a phenomenon which is well known in biology. Lepeschkin *(9)* emphasizes that some seeds can stand heating to 120° C. when dry, but when saturated with moisture they are killed at 60 to 70° C. In peas, also, the most striking effect of temperature upon change in crystal structure, lies between 60 and 70° C.

In spinach, the changes in crystal shape are evident at somewhat lower temperatures. In comparison with the shapes which raw spinach exhibits (Fig. 13) changes can be shown by heating only to 50°. After heating to 60° C. the shapes already look very much like those which may be obtained between 70 and 80° C. Fig. 14 shows the result of heating of spinach to 80° C. This result brings up the question of the desirability of testing whether spinach may not be blanched at 60° just as effectively as at higher temperatures, thus protecting the desirable constituents more efficiently.

Green beans similarly show corresponding changes in crystal form in stepwise heating, with the greatest change occurring between 50 and 60° C.

Corn similarly shows great changes in crystal shape between the raw and the cooked condition (Figs. 15, 16).

In general, it is possible to state that the temperature, rather than the time of heating determines the shape of the crystal pattern which may be formed by a given product. The most striking change lies mainly between 60 and 70°, or at the temperature at which most living substances are killed, and proteins coagulate. The changes in shape resulting from heating are always essentially similar, in that the specific shapes disappear, and mostly simply, smooth needle-like crystals result.

In contrast with heating, freezing brings about much smaller changes in shape, which are most easily recognizable by comparison of raw fresh with frozen fresh product. The characteristic branching of the raw potato extract crystals (Fig. 2) may still be recognized in the fresh frozen potato (Fig. 17), but they are more frail and fewer in number. The entire pattern appears simplified.

This same weakening and simplification of pattern may also be found in other fresh frozen products, for example, fresh frozen asparagus (Fig. 18) compared with fresh asparagus (Fig. 9). The typical shapes of raw asparagus are still obtained after freezing, but they are larger and branch more sparingly. The same relationships also exist between raw frozen peas (Fig. 19) and raw fresh peas (Fig. 11). Here the terminal shapes became broader and partially dissolved. The characteristic branching and pointed ends of raw spinach (Fig. 13) may still be distinctly seen in raw frozen spinach (Fig. 20), but they are weaker and more sparing. Raw frozen corn (Fig. 21) similarly shows a simplification and widening of the crystals in comparison with raw corn (Fig. 15).

In general, it may be stated that freezing of products does not bring about the disappearance of the characteristic crystal forms of the raw products, as does heating, but these forms appear weakened and simplified as a result of freezing. From this it may be concluded that freezing has a more protective effect than heating (or blanching). Thus the blanching, usually done in preparing vegetables for freezing, has the greatest effect in processing.

Freezing in combination with blanching yields forms which can hardly be distinguished from the merely blanched products, for example, blanched frozen asparagus (Fig. 22) compared with that blanched only (Fig. 10).

The drying of products effects varying degrees of changes in the crystal pattern, depending upon the degree of drying. With slight drying, as sometimes occurs during storage for several days at room temperature, there is a weakening of the crystallization patterns. The number and markings on the characteristic individual shapes become fewer. Thus, for example, potatoes at the end of their storage period show frailer forms when compared with potatoes soon after harvest. Complete drying of products yields mostly narrower, markedly changed crystal forms.

After treatment with acids, for example, acetic acid, the characteristic crystal forms do not appear. Only scattered markedly changed, narrow microforms appear.

Following a suggestion by R. E. Liesegang (Frankfurt), the experiments were extended by adding gelatin to the extracts. It was shown that gelatin alone gives crystallization forms (Fig. 23). The addition of gelatin to the extracts results in a superposition of the gelatin forms over those of the extract, for example, gelatin + potato (Fig. 24).

Some other substances, for example starch, also give distinct crystallization patterns.

Varietal differences in products are likewise expressed in small differences in crystal shape.

It is noteworthy that broader microforms are generally formed from raw vegetables and grains, while different fruit varieties only yield very narrow crystallization patterns.

Animal products, for example milk, also yield distinct crystallization forms.

An explanation of these crystallization forms is quite difficult. In general, it may be said that the above described species specific microforms are determined by the combined effects of the crystallization forces of cupric chloride, and the forces produced by the specific biological forms. Crystallographically, these forms may be related to the crystallizations designated as "somatoide Bildungsformen" by Kohl-

schütter *(10)*. Vogelsang *(11)*, who first described such forms exhaustively, considered them to be precursors of crystals, and therefore spoke of crystal embryos, or crystallites. Kohlschütter, on the other hand, compares them with organic structures, in regarding them as "organized, physical-chemical entities." He interprets them as "inorganic organisms" and believes that "in the last analysis, probably the same forces which function in the formation of somatoid forms, also act in the formation of organisms." In explanation of these forms, there are only hypotheses, which will not be discussed more fully here.

SUMMARY

With the aid of a crystallographic method, new characteristic microforms were found, which have characteristic shapes depending upon the species of tissue used. The changes which these products undergo as a result of heating, freezing, drying, and chemical treatment are expressed by different typical changes in these crystallization patterns.

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PLATE I

All figures in 45 diameter magnification

FIG. 1. $CuCl_2$ without additions.

FIG. 2. Raw potato.

FIG. 3. Potato, cooked 1 min.

FIG. 4. Potato, cooked 16 min.

PLATE II

FIG. 5. Potato heated to 50° C.

FIG. 6. Potato heated to 60° C.

FIG. 7. Potato heated to 70° C.

FIG. 8. Potato heated to 80° C.

PLATE III

FIG. 9. Raw asparagus.

FIG. 10. Asparagus heated to 80° C.

FIG. 11. Raw peas.

FIG. 12. Peas heated to 80° C.

PLATE IV

FIG. 13. Raw spinach.

FIG. 14. Spinach heated to 80° C.

FIG. 15. Raw maize.

FIG. 16. Cooked maize.

PLATE V

FIG. 17. Potato, frozen raw.

FIG. 18. Asparagus, frozen raw.

FIG. 19. Peas, frozen raw.

FIG. 20. Spinach, frozen raw

PLATE VI

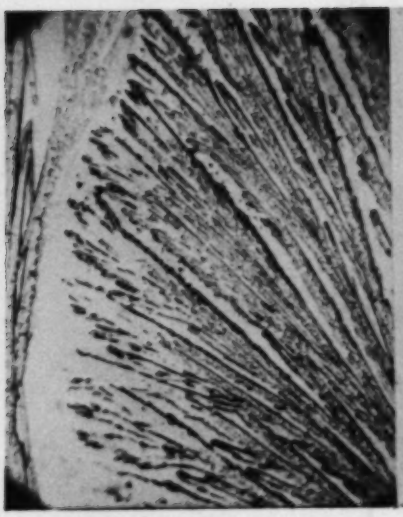

FIG. 21. Maize, frozen raw.

FIG. 22. Asparagus, blanched and frozen.

FIG. 23. Gelatin.

FIG. 24. Gelatin + raw potato.

FOOD VALUE OF SOYBEAN PROTEIN AS RELATED TO PROCESSING

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It has been previously established that appropriate heat treatment results in improvement in the nutritive value of the protein in soybeans. The authors have studied by rat-feeding experiments the growth-promoting value of soybeans subjected to various forms of heat treatment or to alcohol or hexane extraction, the former solvent having been found to be a better debittering agent.

Exposure to steam at atmospheric or 15-lb. pressure caused improvement in nutritive value which reached a maximum but diminished on further heating. Dry heat (120°C) was ineffective. Hexane- and alcohol-extracted soybean flakes after steam treatment had substantially the same optimal nutritive value.

Soybean flakes prepared by the commercially popular hexane-extraction procedure retain bitter principles which, to some extent, discourage the use of the flakes in food preparations. The Northern Regional Research Laboratory is engaged in the development of a method of producing alcohol-extracted soybean flakes which, owing to the removal of the bitter principles in the alcohol extraction, have a blander and hence more acceptable taste than the hexane-extracted material, according to Belter, Beckel, and Smith *(2)* and Beckel and Smith *(1)*.

At the suggestion of the Northern Regional Laboratory, this Laboratory has undertaken a study of the comparative values of hexane- and alcohol-extracted soybean flakes as determined by feeding tests with rats. The nutritional inadequacy of soybean protein in the unheated state and its improvement by heating or supplementation with methionine are well established, Hayward and Hafner *(5)*; Hayward, Steenbock, and Bohstedt *(6, 7)*; and Wilgus, Norris, and Heuser *(11)*. Hayward *(4)* demonstrated that unheated alcohol-extracted soybean oil meal and unheated hexane-extracted soybean oil meal, when fed to rats, had no greater nutritive value than the completely unsatisfactory raw soybeans, but he did not compare the optimally heated samples. Experiments were carried out at this Laboratory to determine the optimum conditions of heat treatment so that the hexane- and alcohol-extracted flakes could be compared at their maximum nutritional value. These experiments are presented here, since they show in some detail the change in nutritional value as a function of kind and extent of heating.

EXPERIMENTAL PROCEDURE

Albino rats of the Sprague-Dawley strain were obtained from our stock colony, which is maintained on a diet of Purina Dog Chow Checkers supplemented with cod-liver oil and liver. Litters of rats, about 40 days of age, and ranging in weight from 70-100 grams per rat, were used since they had been found to give less variable response to changes in dietary protein than did weanling rats. Each group contained 10-12 rats, with equal numbers of males and females, housed in two cages. The experimental ad libitum feeding period was six weeks unless otherwise stated. A complete food consumption record was kept for each group by weighing the residual food in the cup and amount of food added at 2 to 3 day intervals. The individual rats were weighed weekly. Results were calculated as grams of weight gained per

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[b] Bureau of Agricultural and Industrial Chemistry, Agricultural Research Administration, U. S. Department of Agriculture.

gram of protein eaten. The level of crude soybean protein (% nitrogen × 5.71[e]) was 14.1% in the heating experiments (Exp. 1 and 2) and 14.6% in all others. Casein was also fed at 14.6% crude protein (% nitrogen × 6.38). This level of crude protein was selected because it approaches the practical levels used in animal husbandry and at the same time is low enough to give adequate differences as a function of processing. Each experimental diet contained, in addition to its particular protein supplement, the basal mixture representing 50% of the complete diet and an equal weight mixture of starch and sucrose for the remainder. The basal mixture contained, as percent of the complete diet: cottonseed (Wesson) oil,

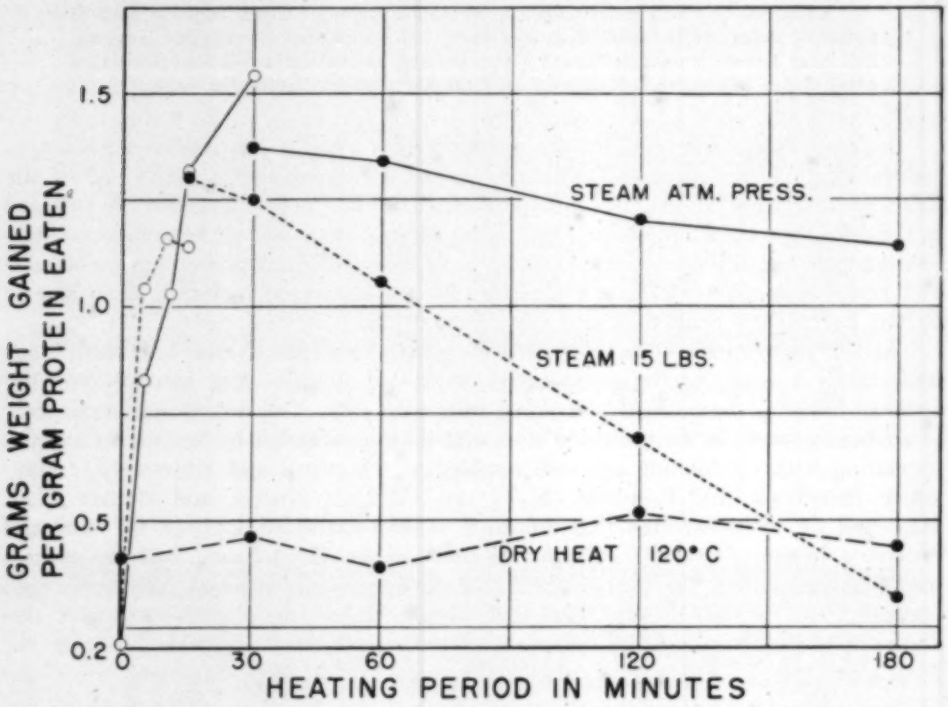

FIG. 1. Effect of type and extent of heat treatment on nutritional value of soybean protein.[1]

● Experiment 1 ○ Experiment 2

———————Steam at atmospheric pressure

- - - - - - - - - - - -Steam at 15 lb. pressure

— — — — — — —Dry heat 120° C.

[1] Test period, 42 days; 12 rats per group; 80 grams average initial weight.

5; cod-liver oil (U.S.P.), 2; vegetable oil concentrate having 34% tocopherols, 0.05; choline chloride, 0.05; brewers' yeast, 2; salt mixture (McCollum's No. 185 + trace elements), 4; corn starch 18.5; sucrose, 18.5; and as mg. per 100 g. complete diet: thiamine 0.2; riboflavin, 0.5; pyridoxine, 0.2; calcium pantothenate, 2.5; and niacin, 1.0.

[e] As recommended by D. B. Jones *(8)*, which has been a generally accepted value, although recent nitrogen data, Smiley and Smith *(10)*, on extracted soybean protein indicate that it may be more nearly 5.9. Variations owing to variety, nonprotein nitrogen, and fractionation of the proteins make assignment of an absolute value difficult and of little value in this report where practically all comparisons are between samples of the same protein-soybean.

HEATING EXPERIMENTS

Ground raw soybeans were exposed in shallow (1 inch) trays for various periods of time to the following three types of heat treatment: dry heat (120° C.) in a Proctor-Schwartz circulating air oven; steam at 15 lb. pressure (120° C.) in a steam-jacketed autoclave with steam in sample chamber; steam at nearly atmospheric pressure in a steam-jacketed autoclave with 5 lb. steam in jacket blowing into sample chamber with all exhaust valves open and chamber door ajar.

The data relating nutritional value to length of exposure for the three types of heat treatment are plotted in Figure 1. With 15 lb. steam there was a sharp rise to a maximum value at 10 to 15 minutes, beyond which a gradual decrease occurred. The steaming at atmospheric pressure gave a slightly higher maximum at 30 minutes

TABLE 1

Supplementary effect of amino acids on value of overheated soybean protein[1]

Diet No.	Treatment and supplements to hexane-extracted flakes	Exper.	Average gain, g./rat/day	Feed consumption g./rat/day	Efficiency g. gain/g. protein eaten	
1	None	a	1.01	9.7	0.69	
		b	0.75	9.2	0.54	0.67
		c	1.30	11.0	0.78	av.
2	15 lb. steam, 15 min.	a	2.67	12.7	1.38	
		b	2.52	12.0	1.38	1.31
		c	2.07	11.8	1.16	av.
3	15 lb. steam, 3 hours	a	0.70	10.5	0.44	
		b	0.74	10.4	0.47	0.52
		c	1.07	11.1	0.64	av.
4	15 lb. steam, 3 hr. + 0.3% leucine	a	0.84[2]	11.1	0.49	
5	15 lb. steam, 3 hr. + 0.3% lysine	a	0.33[3]	9.5	0.22	
6	15 lb. steam, 3 hr. + 0.5% leucine + 0.4% lysine	b	0.25[4]	11.4	0.14	
7	15 lb. steam, 3 hr. + 0.3% methionine	c	1.64[5]	10.9	0.98	
8	15 lb. steam, 3 hr. + 0.3% methionine + 0.5% leucine	c	1.63[5]	10.9	0.94	
9	15 lb. steam, 3 hr. + 0.3% methionine + 0.4% lysine	c	2.26[5]	12.8	1.11	
10	15 lb. steam, 3 hr. + 0.3% methionine + 0.5% leucine + 0.4% lysine	a	2.72	11.5	1.61	1.47
		b	3.00	13.7	1.50	av.
		c	2.68[5]	12.5	1.31	
11	15 lb. steam, 3 hr. + 10 essential amino acids[6]	b	3.10	11.2	1.48	
12	Casein	a	3.20	13.7	1.53	
		b	2.88	12.9	1.47	1.54
		c	3.00	12.1	1.63	av.

[1] 8-10 rats per experimental group, 86-90 grams average initial weight, 28-day test period, unless otherwise stated. All numerical values in the table represent means of the male and female averages.

[2] 25 days only.

[3] 15 days only.

[4] For 7 days only, immediately following 14 days on Diet No. 10.

[5] In Experiment c, an analysis of variance of the combined male and female data indicates that the difference between groups 7 and 10 or 8 and 10 is highly significant (1% level of probability); the difference between groups 9 and 10 is barely significant at a 5% level of probability.

[6] In percent of diet: dl-methionine, 0.3; l-lysine HCl, 0.5; l-leucine, 0.4; dl-isoleucine, 0.5; l-histidine HCl, 0.4; dl-tryptophane, 0.2; dl-phenylalanine, 0.4; dl-threonine, 0.3; dl-valine, 0.4; l-arginine HCl, 0.2.

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TABLE 2

Comparison of values[1] of hexane-, hot alcohol-, and cold alcohol-extracted soybean flakes

Group No.	Protein supplement (14.6% crude protein)	Experiment 1 [2]			Experiment 2 [3]		
		Average gain g./rat/day	Feed consumption g./rat/day	Efficiency g. gain per gram protein eaten	Average gain g./rat/day	Feed consumption g./rat/day	Efficiency g. gain per gram protein eaten
1	Unextracted soybeans, steamed at atmos. pressure 30 min.	1.95	10.7	1.20			
2	Hexane-extracted soybean flakes	0.52	9.0	0.38	0.65	9.0	0.47
3	Hot alcohol ext. soybean flakes	0.53	8.9	0.39	0.83	10.0	0.54
4	Cold alcohol ext. soybean flakes	....	....	....	0.96 [4]	10.5	0.60
5	Hexane-extracted soybean flakes steamed at atmos. press. 30 min.	1.93	10.2	1.24	2.35	11.2	1.38
6	Hot alcohol ext. soybean flakes steamed at atmos. press. 30 min.	1.81	10.7	1.11	2.39	11.6	1.36
7	Cold alcohol ext. soybean flakes steamed at atmos. press. 30 min.	....	....	....	1.97 [4]	11.4	1.14
8	Hexane ext. flakes, steamed + hexane-ext. soybean oil	1.92	10.5	1.20	2.22	11.2	1.31
9	Hot alcohol ext. flakes, steamed + hot alcohol ext. soybean oil	1.95	11.3	1.13	2.35	12.6	1.23
10	Casein	2.64	11.7	1.48	2.91	13.6	1.41

[1] All numerical values in the table represent means of the male and female averages.

[2] Test period, 41 days; 12 rats/group; 88 g. average initial weight.

[3] Test period, 42 days; 6 males, 5 females/group; 91 g. average initial weight.

[4] On the basis of an analysis of variance applied to the combined data on male and female weight gains, the differences between groups 2, 3, and 4 are not significant at a 5% level of probability; differences between groups 5, 6, 8, and 9 are also not significant. In Experiment 2, the small difference between groups 6 and 7, however, is statistically significant but not readily explainable.

and less loss on prolonged heating. Dry heat had no beneficial effect. It was also found that dry heat for 30 minutes caused no destruction of the potential value, since steaming such a sample resulted in maximum improvement, i. e., from 0.5 for the dry-heated sample to 1.1 grams gain per gram protein for the dry-heated and steamed material.

Further feeding experiments were carried out on the destructively heated (15 lb. steam, 3 hours) soybean sample in order to determine the amino acids involved. The results, summarized in Table 1, demonstrate that the destructive effect of 15 lb. steam over a relatively long period produces a deficiency of methionine primarily and a definite but not as serious deficiency of lysine and leucine. When methionine was removed from the combination supplement of methionine, leucine, and lysine, the growth rate immediately dropped to a very low value.

Since this paper was written, work of a similar nature with chicks has been published by Clandinin et al. *(3)*, which is in substantial agreement with our results. They found that a practical type of chick ration, with approximately 60% of the total protein furnished by overheated soybean oil meal, was deficient in available lysine and methionine for optimum growth of chicks. Their experiments also indicated an optimum cooking time of 4-15 minutes with steam at 15 lb. pressure. McGinnis and Evans *(9)* have found that the protein efficiency in chick diets of destructively heated soybean meal was increased about 30% by addition of a combination of methionine, cystine, and lysine.

COMPARISON OF HEXANE- AND ALCOHOL-EXTRACTED SOYBEAN FLAKES

Samples used in this comparison were prepared by the Northern Regional Research Laboratory on a laboratory scale from a uniform lot of soybeans of the Dunfield variety. In the case of the hexane and hot alcohol extraction, the extractives were freed of solvent and sent to us with the flakes. The hexane-, hot alcohol-, and cold alcohol-extracted flakes contained 37.6, 44.9, and 49.1% crude protein, respectively, while the raw whole soybean from which they were prepared had 31.2% crude protein.

For this series of experiments, the basic level of cottonseed (Wesson) oil in all diets was raised from 5 to 9%, or approximately equal to the amount of oil furnished by the level of unextracted soybeans fed. Soybean oil, when present in the diet, replaced an equal weight of Wesson oil, so that all diets contained the same amount of oil. The hexane or the alcohol extractives were combined with the flakes in the diets in the proportions originally present in the soybeans.

Table 2 presents the results obtained in two separate feeding experiments on the above samples. Comparisons were made of the hexane- and alcohol-extracted flakes in the unheated state, after steaming for 30 minutes, and steamed but with the solvent extractives added in replacement of an equal weight of cottonseed oil. In no case was the difference between hexane- and alcohol-extracted flakes statistically significant. Replacement of cottonseed oil by soybean oil had no apparent influence on the over-all nutritional value. The steamed extracted flakes and the steamed unextracted soybeans also gave essentially the same value.

SUMMARY

The effect of variations in heat treatment and solvent extraction on the value of soybean protein was studied by means of feeding experiments with rats. Treatment of raw soybeans with steam at 15 lb. pressure gave a product of maximum nutritional value after 10-15 minutes. On heating beyond this optimum time, there was a gradual decrease owing to the production of a deficiency of methionine, lysine, and leucine. Steam at about atmospheric pressure for 30 minutes resulted in a slightly

higher maximum, and less destruction took place on prolonged heating. Dry heat had no effect.

Nutritional values for rats of hexane- and alcohol-extracted soybean flakes prepared from a uniform source were essentially the same and, with optimum heat treatment, equal to that of the optimally steamed, unextracted soybeans. Inclusion in the diets of the oils extracted by the two solvents had no influence on the results.

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EFFECT OF HIGH TEMPERATURE STORAGE ON THE RETENTION OF NUTRIENTS IN CANNED FOODS [a]

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A study of the stability of the vitamins occurring to an appreciable extent in thirteen representative canned foods (apricots, orange juice, tomato juice, carrots, peas, spinach, lima beans, green beans, whole kernel yellow corn, Army Spread, cheese spread, dried whole milk and evaporated milk) used in Army rations was made through an 18 months' storage period at 70°, 90°, and 100°F.

Of the vitamins studied, thiamine and ascorbic acid were the most labile on storage, the greatest losses occurring at the higher storage temperatures. The niacin, folic acid, vitamin A and carotene content of the canned foods showed no appreciable losses at any storage temperature. The riboflavin content of the canned fruits and vegetables showed marked increases on storage at all three temperatures (25-200%) whereas the riboflavin content of the dairy products remained at their initial level with the exception of Army Spread which declined somewhat.

INTRODUCTION

In 1942 the National Canners' Association in cooperation with the Can Manufacturer's Institute, sponsored a Nutrition Program *(3)* to study the nutritive value of canned fruits and vegetables as affected by methods of processing, by methods of preparation (Army, home, and restaurant) and by storage time and temperatures encountered in the usual commercial channels of supply. This type of information was essential for intelligent planning, evaluation and rationing of human dietaries during the anticipated emergency and food shortages.

In August 1943, temperature studies on foods in field storage, were conducted in the California desert by the Subsistence Research and Development Laboratory in cooperation with the American Can Company and the Continental Can Company Research Departments to determine the laboratory temperatures simulating elevated storage temperatures encountered in some overseas theaters of operation. Temperatures ranging from 95 to 103° F. were found within stacks of canned foods stored in the open with and without tarpaulins. While food in the top of a stack, particularly if it was covered with a tarpaulin, may have reached temperatures higher than 100° F., the average of the stack was in the range of 95 to 100° F. As a result of this study the 100° F. temperature was chosen as a suitable approximation for the study of deteriorative effects of storage in hot dry climates *(6)*.

The Nutrition Program described above with the exception of one storage study conducted at 110° F. on three foods *(8)* did not furnish information on the exact nature and extent of deterioration occurring in canned foods under storage at elevated temperatures similar to the extremes encountered in overseas theaters of operation. On 25 October 1944, following preliminary correspondence, a meeting of the Subsistence Research and Development Laboratory personnel was held with the representatives of the National Canners' Association, American Can Company and Continental Can Company Research Departments to discuss and make tentative plans for a supplementary study on canned foods to fill the gaps in the existing storage picture. The plan of the cooperative study which was thus evolved is described below.

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[a] A separate paper on the effect of high temperature storage on the acceptability of canned foods is to follow by Dove et al.

[b] At present with Libby, McNeill and Libby, Blue Island, Illinois.

[c] At present with Department of Bacteriology, University of Colorado Medical School.

EXPERIMENTAL PROCEDURE

Selection of Samples: The choice of canned foods to be included in this study was based upon several considerations, namely:

a. order of volume production in million cases for 1943 for the various canned foods.
b. order of percentage government reservation of each canned food product.
c. order of contribution of the various nutrients by the foods selected on the basis of a and b.
d. order of pH ranges of canned foods.

Thus the products finally selected (apricots, orange juice, tomato juice, carrots, peas, spinach, green beans, whole kernel yellow corn, and lima beans) represented the most generally used canned foods combining the variables in pH and vitamin content as is shown in Table 1.

Representative canned dairy products and canned meat items were also to be included as part of this study in order to make available complete information on the nutritive stability and palatability of all canned ration constituents. Army Spread, cheese spread, evaporated milk and dried whole milk powder were chosen for the dairy products. The canned meat study was conducted by the Armour Laboratories and the description of the samples and the results obtained may be found in Interim Report No. 2 Vitamin B Complex, Stability *(2)*.

Sample Collection: The quality of the samples chosen represented that of the individual products purchased in the greatest quantities by the QMC. In order to avoid the necessity of tracing back history on samples and unnecessary storage, the samples for study were to be packed during the next canning season and the variety, growing conditions, and the details of the processing methods recorded. Retail size cans, (No. 2 or 2½) except in the case of the canned dairy products, in which regular Army size ration cans [d] were employed, were numbered in the order in which they were filled and closed, and after processing were sorted and coded for product, storage time and storage temperature. The sorting assured the inclusion of cans from the various parts of the pack in the initial and subsequent examinations.

Analyses and Storage: Inspection of 6-8 cans of the food was made for net weight, vacuum and headspace measurements and pH. Two additional cans [e] were used for food acceptance tests and 6 cans were retained for the initial vitamin assays. Division of the remaining cans of the 244 to 288 pack was made for storage at 70, 90, 100° F. and for the Florida warehouse.[f] Eight cans, two for acceptability tests and 6 for vitamin assay, were allocated for each storage condition at each examination. Florida warehouse samples following 12 to 18 months' storage were included because it was felt that the conditions encountered here might be more representative of those found in some parts of the Pacific theater of operation than those under controlled laboratory storage. Each food was to be assayed for those vitamins of which it contributed in one serving, amounts equal to or greater than 1/10 of the minimum daily requirements set by the Federal Food and Drug Administration for use in labelling foods.

Table 2 indicates the determinations to be made on each product; Table 3 contains the scheduled examinations for the various vitamins. It is seen that more

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[d] Army Spread and cheese spread were packed in 3¾ oz. cans; evaporated milk in 14½ oz. cans and dried whole milk powder in 1 lb. cans.

[e] In the case of Army Spread and cheese spread, 6 cans instead of 2 were used for food acceptance tests.

[f] The American Can Company facilities were used for the 70° F. storage and the incubators at the Subsistence Research and Development Laboratory were employed for the 90 and 100° F. storage. The Tampa, Florida, warehouse of the American Can Company was used for simulated field conditions.

TABLE 1

pH and Vitamin Variables in Canned Foods Selected for High Temperature Storage Study

pH	Product	Variety	Carotene	Thiamine	Riboflavin	Niacin	Ascorbic Acid	Vit. A
			mg. per 100 g.	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*	*mg. per 100 g.*
3.90	Apricots	Blenheim	1.06	.026	low	low	6.70	.....
3.56	Orange Juice	Valencia 01	low	.058	low	low	41.1	.....
3.70	Orange Juice	Valencia 02	.120	.083	low	low	43.2	.....
4.35	Tomato Juice	Rutgers	.480	.058	.033	.079	13.0	.....
5.37	Carrots	Chantenay	7.40	low	low	low	low	.....
5.70	Green Beans	Refugee	.110	.024	low	low	2.20	.....
5.40	Spinach	Viroflay	3.20	low	.080	low	24.0	.....
6.78	Whole Kernel Corn, Yellow	Golden Cross Hybrid and Silver Cross	.030	low	.028	.890	low	.....
6.05	Lima Beans	Clark Green Seed	low	.031	.038	.540	14.2	.....
6.30	Peas	Prince of Wales	.230	.127	.048	1.14	11.6	.....
6.50	Army Spread	C. Q. D. Spec. No. 32C	.192	.....	.160	.....	.....	.810
5.82	Cheese Spread	C. Q. D. Spec. No. 358	.384	.....	.280	.....	.....	.370
6.40	Dried Milk	C. Q. D. Spec. No. 166A	.237	.....	1.48	.....	.....	.176
6.30	Evaporated Milk	Fed. Spec. CM 371	.106	.....	.320	.....	.....	.046

TABLE 2

Analytical Schedule

Vitamin	Months										
	0	½	1	2	4	6	8	10	12	15	18
Vitamin A and Carotene	×			×	×	×	×	×	×	×	×
Thiamine	×			×	×	×	×	×	×	×	×
Riboflavin	×				×		×		×	×	×
Niacin	×				×		×		×	×	×
Vitamin C	×	×	×	×	×	×	×	×	×	×	×
Food Acceptance	×		×	×	×	×	×	×	×	×	×
Food Inspection	×										

TABLE 3

Analytical Pattern

Product	Vitamin A	Carotene	Thiamine	Riboflavin	Niacin	Vitamin C
Apricots		×	×			×
Beans, Green		×	×			×
Beans, Lima			×	×	×	
Carrots		×				
Whole Kernel Yellow Corn		×		×	×	
Peas		×	×	×	×	×
Orange Juice		×	×			×
Spinach		×		×		×
Tomato Juice		×	×	×	×	×
Army Spread	×	×		×		
Cheese Spread	×	×		×		
Milk, Evaporated	×	×		×		
Milk, Dried, Whole	×	×		×		

frequent intervals of assay were used for the less stable nutrients (ascorbic acid, thiamine, carotene, and vitamin A) than for the more stable ones (riboflavin and niacin). Although the total storage time was planned for a period of 18 months at each temperature, it was agreed to terminate the vitamin assays on a food at the times at which its deterioration in palatability reached the point of unacceptance.

The method of preparation of the slurries for vitamin assay [g] was similar to that employed in the NCA-CMI Nutrition Study *(3)*. The average solid and liquid contents of the 6 cans were determined. Composite samples (200 g.) containing proportionate parts of liquid and solid were then blended into a homogeneous mixture or slurry with 1 ml. of $CHCl_3$ and equal portions of alcoholic potassium hydroxide containing 0.5% KOH for carotene assay or with 0.2 N H_2SO_4 for "B" vitamin assay. For the ascorbic acid assay the composite was blended with an equal weight of 3% metaphosphoric acid in 8% acetic acid. In the case of apricots and spinach, the entire contents of the 6 cans were blended and aliquots of the pureed mixtures were used in the preparation of slurries for the various assays. The carotene and B vitamin slurries which were sent to other laboratories for assay were placed in pint jars, and the slurry covered with toluene. Three such slurries were prepared for each sample, two slurries being sent to the laboratory, the third one being kept as replacement in the event of breakage or leakage. Duplicate analyses were made for each determination.

[g] The 6 can composite of the canned dairy products, however, was prepared by thorough mixing and sampling directly for vitamin A and carotene assay. Slurries made by blending 200 g. of samples of the 6 can composite with 200 g. of 0.2 N H_2SO_4 were then made and forwarded for riboflavin assay.

Because of the general interest in folic acid, samples of canned vegetables known to contain significant amounts of the nutrient (spinach, green beans, and carrots)[h] were sent to the University of Wisconsin for assay initially and at various intervals of storage. Two cans were assayed individually and at each successive examination employing proportionate amounts of liquid and solid. Folic acid content of canned spinach was determined at 70, 90, and 100° F. storage following 2, 4, 6, and 12 month intervals, that of green beans following 2 and 4 months and that of carrots after 4 months.

The methods of assay[i] used for the various determinations were as follows:

Thiamine—Thiochrome procedure of Conner and Straub *(4)*.

Riboflavin and niacin—Microbiological techniques using *L casei* and *L arabinosus* respectively *(18, 19)*.

Folic acid—Microbiological technique employing *S faecalis R* and *L casei (13)*.

Ascorbic acid—Visual titration procedure with 2,6-dichlorophenolindophenol *(21)*.

Carotene—Extraction with foaming mixture and chromatographic separation through MgO-Supercel mixture 1-4 *(22)*.

Vitamin A—Carr-Price $SbCl_3$ reaction on unsaponifiables corrected for blue color due to carotene *(12)*. Chromatographic separation was used to determine carotene in dairy products *(22)*.

Food acceptance was determined by taste test panels *(5)*.

RESULTS

Vitamin Stability in Stored Canned Foods:

Thiamine: Although there was a marked fluctuation in the thiamine values obtained on aliquots from the 6 can composite assayed at the various time intervals, certain trends in thiamine stability were evident. Figure 1 demonstrates the relationship between thiamine retention and storage time and temperature.[j] It is seen that on the whole, the loss of thiamine in canned foods increased as the length of storage and storage temperature was increased. At 70° F. storage, at least 80% or more of the initial thiamine content was retained in the samples of tomato juice, orange juices, lima beans and peas through the 18 months' storage period. The samples of apricots and green beans, however, retained only 60% thiamine under the same storage conditions, the bulk of the loss occurring prior to 6 months' storage with little or no additional losses following this period. At the higher storage temperatures canned apricots suffered the greatest thiamine losses but temperature differences were not clear-cut until the 8th-10th month of storage. Following this period the retention at 90° and 100° F. was about 50 and 40% respectively, declining to 35 and 20% respectively at the end of the 18 months' storage period. Marked temperature retention differences were also not observed for canned green beans in which approximately 50% of the initial thiamine content disappeared after 6 months' storage and remained at that level throughout the duration of the storage test at 90 and 100° F. Canned peas and lima beans after storage for 4 months' showed clear-cut temperature differences, approximating 55 and 35% retention after 18 months' storage at 90 and 100° F. storage respectively.

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[h] The assay of these items was suggested by Dr. Elvehjem.

[i] Distribution of the assays among the cooperating laboratories was as follows:
Vitamin A and carotene in dairy products—QMF & CI.
Carotene and thiamine—Continental Can Company.
Ascorbic acid—American Can Company.
Riboflavin and niacin—National Canners' Association—Following initial and 4 months' analyses, assays for riboflavin and niacin were taken over by American and Continental Can Co. respectively due to loss of personnel at NCA.
Taste tests—Food Acceptance Research Branch, QMF & CI.

[j] Regression lines for thiamine were fitted by the method of least squares.

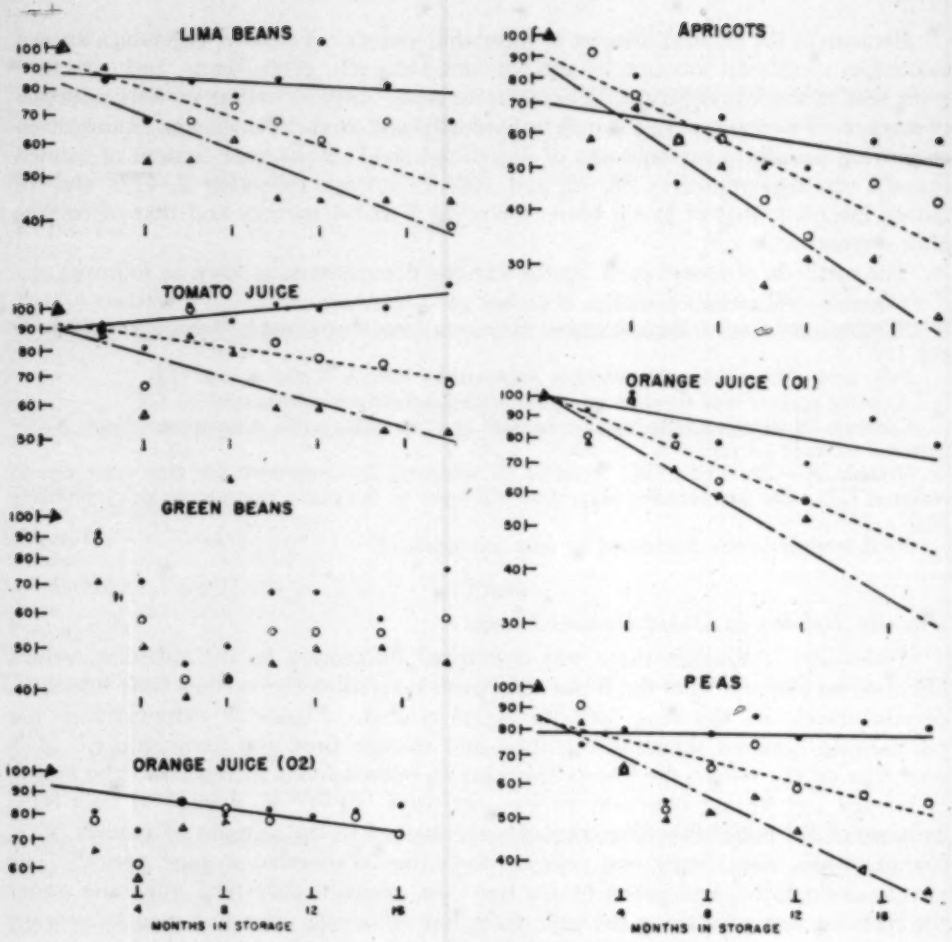

FIG. 1. Percent Retention of Thiamine on Storage.

Retention of thiamine in the canned juices at the elevated storage temperatures compared favorably to the retention in canned peas and lima beans. The tomato juice showed better retention of thiamine than did the orange juice and also more distinct temperature differences at 90 and 100° F. Retention of thiamine in tomato juice following 8 months' storage at these temperatures was approximately 70 and 50% respectively. Assays for thiamine in the samples of orange juice were not carried through the entire 18 months' storage period since palatability, especially at the 100° F. storage temperature, had deteriorated beyond the acceptable range at the 6 to 8 months' storage period. Although one sample of orange juice (02) deteriorated in flavor more rapidly than the other sample (01) it retained its thiamine content somewhat better at both 90 and 100° F. (80 vs. 65% retention at 8 months' storage at 90° F.).

Riboflavin: The riboflavin content of the canned food items studied showed the expected stability on storage at all three temperatures. There was much fluctuation in riboflavin content of the samples assayed at the various scheduled periods and in several of the foods large increases in riboflavin content occurred on storage. See Figure 2.

The dairy products, dried whole milk and cheese spread, showed the least variations in riboflavin content from interval to interval. Retention of riboflavin

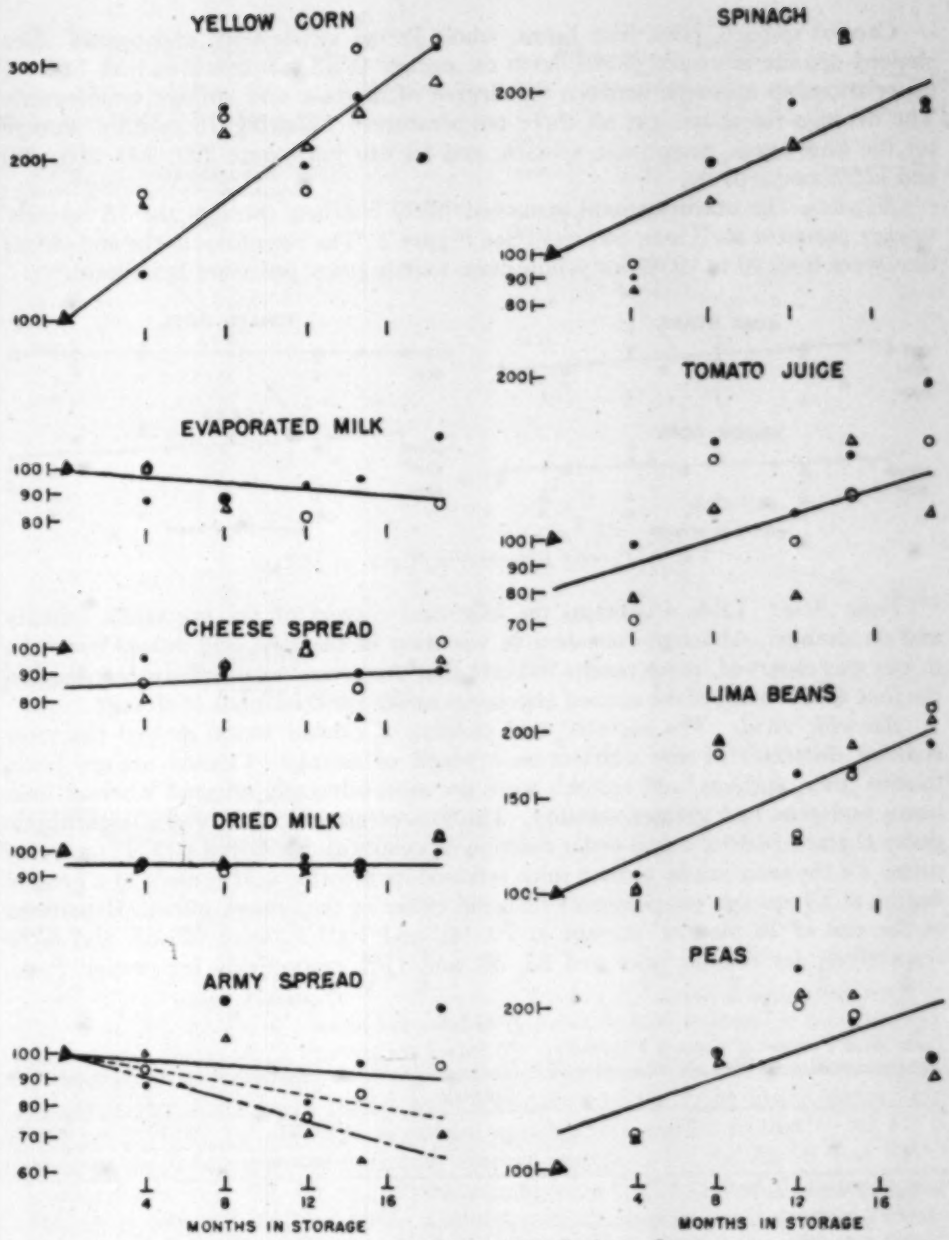

FIG. 2. Percent Retention of Riboflavin on Storage.

in these products was excellent through the 18 months' storage (100% retention) and there were no significant temperature differences observed. The riboflavin retention in evaporated milk and Army Spread, however, declined 10 and 30% respectively at the end of the 18 months' storage period at the elevated temperatures. The palatability of the evaporated milk deteriorated to the point of unacceptability at both 90 and 100° F. following 6 to 8 months' storage. Riboflavin assays of the 100° F. samples were therefore discontinued at this time. The 90° F. samples were assayed throughout the test despite their poor acceptability.

Canned spinach, peas, lima beans, whole kernel yellow corn and tomato juice showed definite increases of riboflavin on storage at all temperatures with little or no relationship apparent between the degree of increase and storage temperature. The average retentions (at all three temperatures) following 18 months' storage for the lima beans, corn, peas, spinach, and tomato juice were 200, 300, 200, 200 and 125% respectively.

Niacin: The niacin content remained fairly constant through the 18 months' storage period at the 3 temperatures. See Figure 3. The retentions at the end of this time were from 90 to 100% for yellow corn, tomato juice, peas, and lima beans.

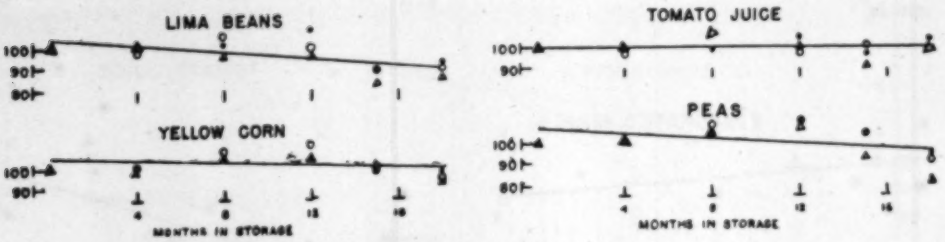

FIG. 3. Percent Retention of Niacin on Storage.

Folic Acid: Table 4 contains the folic acid content of the vegetables initially and on storage. Although considerable variation in the folic acid values from can to can was observed, these results indicate that there was no significant decrease in the folic acid content of the canned vegetables under the conditions of storage.

Ascorbic Acid: The ascorbic acid content of canned foods showed the most striking decreases of any nutrient as a result of storage. Canned orange juice, tomato juice, apricots, and spinach were the most adversely affected whereas lima beans and peas had greater stability. The loss of ascorbic acid was a logarithmic decay characteristic of a first order reaction especially at the 90 and 100° F. temperatures. Of the fruit juices, tomato juice retained its ascorbic acid content to a greater degree at all storage temperatures than did either of the orange juices. Retentions at the end of 18 months' storage at 70, 90, and 100° F. were 92, 65, and 45% respectively for tomato juice and 82, 38, and 11% respectively for orange juice.

TABLE 4

Folic Acid Content of Canned Vegetables. All values are averages of the results from two cans.

		70° F.	90° F.	100° F.
		Micrograms per gram of total solids		
Spinach				
Initial (2 weeks at 70°)	.074			
Stored 2 months		.13	.09	.14
Stored 4 months		.24	.21	.16
Stored 6 months		.17	.14	....
Stored 12 months				.09
Green Beans				
Initial	.05			
Stored 2 months		.02	.01	.01
Stored 4 months		.03	.02	.02
Carrots				
Initial	.024			
Stored 4 months		.06	.05	.05

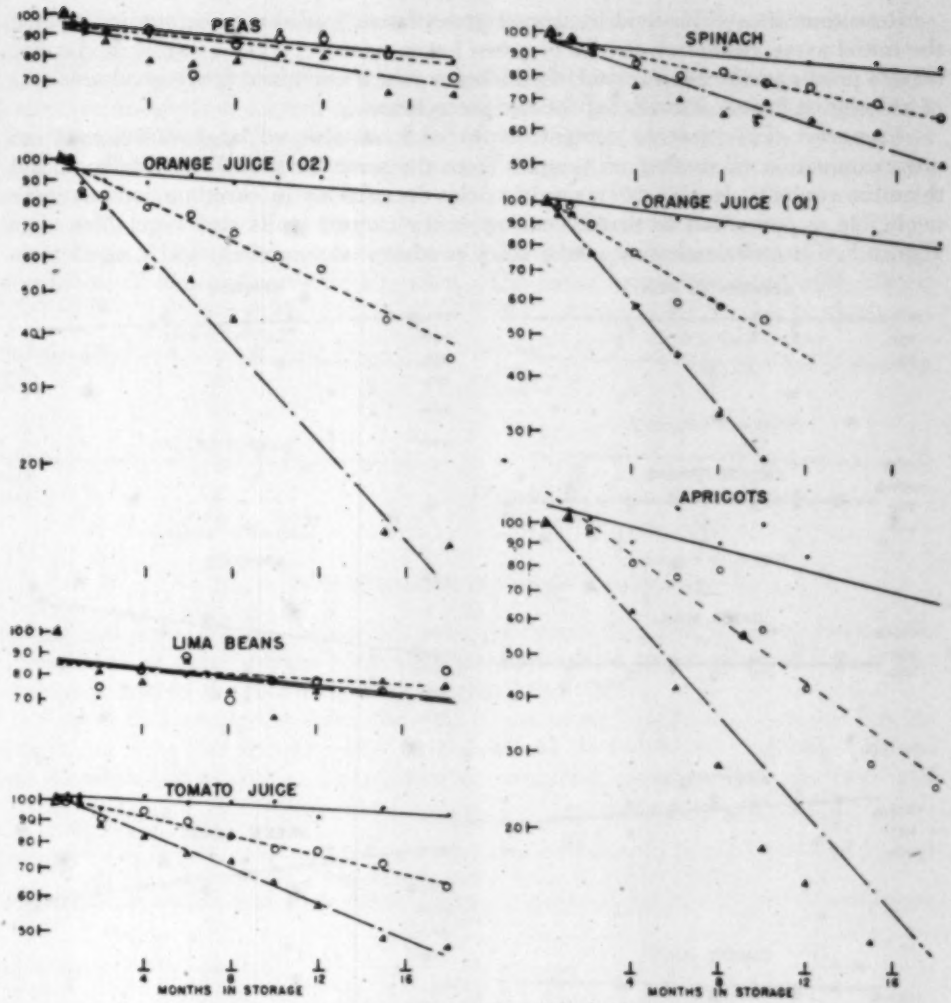

FIG. 4. Percent Retention of Ascorbic Acid on Storage.

Temperature differences were very marked (Figure 4)[k] and fluctuations in assay values during the storage period were minimized by the large losses encountered. The retention of ascorbic acid in canned apricots was similar to that found for the orange juice. At the end of 6 months' storage retentions for the 70, 90, and 100° F. temperatures were 93, 70, and 46% respectively; at the end of 18 months these retentions were 67, 26, and 10%. Canned spinach showed more gradual losses in the early storage period. The retentions following 18 months' storage were 82, 64, and 50% at 70, 90, and 100° F. respectively.

Canned limas and peas, on the other hand, though containing smaller quantities of ascorbic acid initially showed slight losses in ascorbic acid content on storage and not as pronounced temperature differences as noted for the other foods. Canned limas retained approximately 70% through 18 months' storage at all 3 temperatures, whereas canned peas retained approximately 80, 75, and 66% at 70, 90, and 100° F. respectively for the same storage period.

[k] Regression lines for ascorbic acid were fitted by the method of least squares.

Retention of ascorbic acid in canned green beans was also to be studied. Since the initial assay results on the lot of green beans were very low ascorbic acid assay on this product was discontinued. Lima beans which contained fairly good amounts of ascorbic acid were substituted for the green beans.

Carotene: The carotene content of canned foods showed large variations from one examination to another on samples from the same lot as did the riboflavin and thiamine content. In the dairy products the fluctuations in carotene content were negligible as compared to that occurring in the canned fruits and vegetables. See Figure 5. Carotene retention in the dairy products was excellent and ranged from

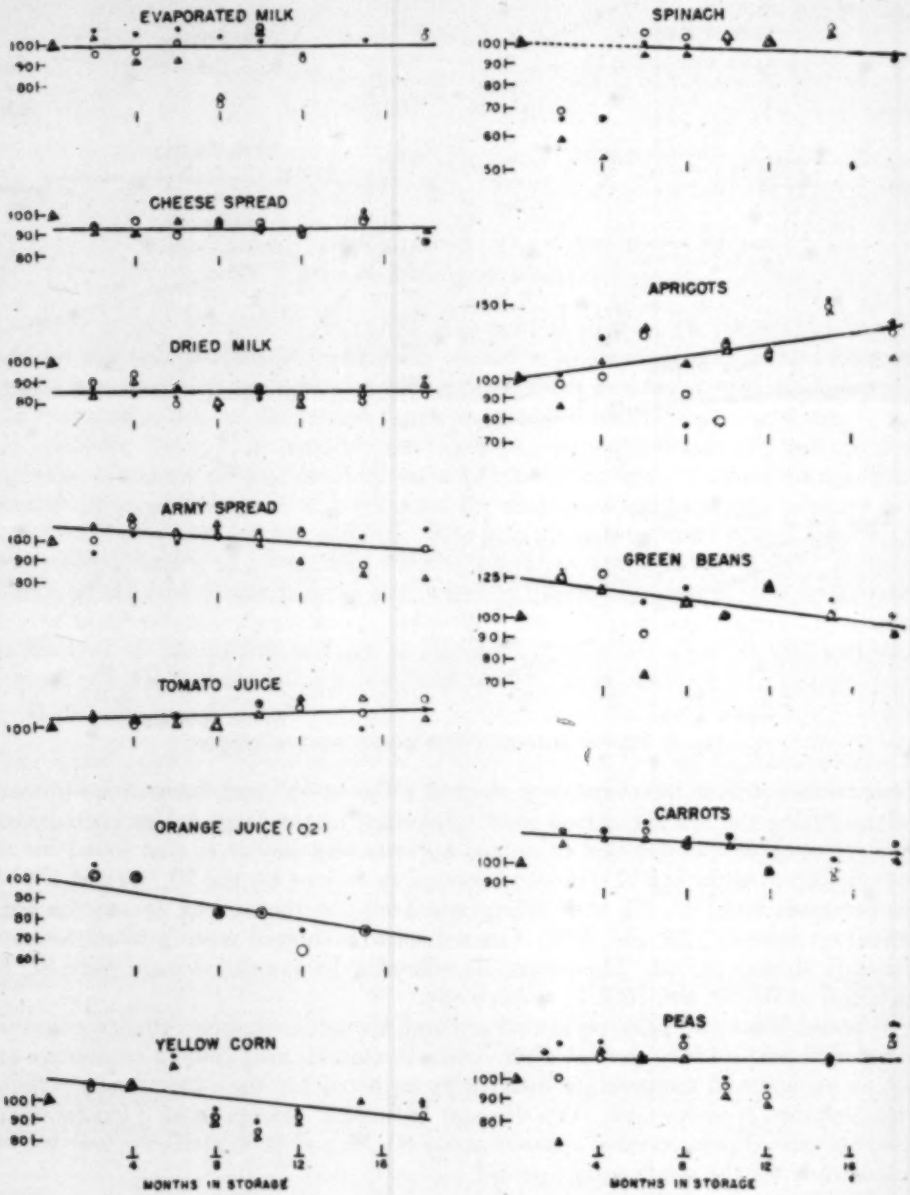

FIG. 5. Percent Retention of Carotene on Storage.

85 to 100% at the end of the 18 months' storage period, the 15% decline occurring in Army Spread.

The carotene of canned fruits and vegetables also had good storage life. Slight increases in carotene content on storage were noted for tomato juice and apricots. Peas, spinach, and carrots retained from 90 to 100% of the initial carotene present at the end of the 18 months' storage period whereas the yellow corn, orange juice, and green beans showed greater losses approximating 15, 25, and 30% respectively at the end of this time. There were no temperature differences observed.

Vitamin A: The vitamin A content of dairy products was unaffected by storage regardless of temperature. See Figure 6. The assay values for dried milk, cheese

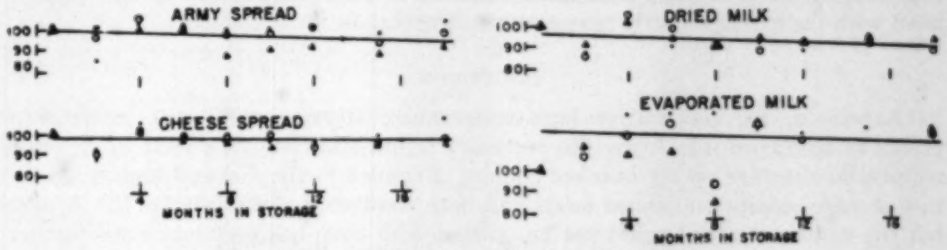

FIG. 6. Percent Retention of Vitamin A on Storage.

spread and Army Spread were much more consistent than that for evaporated milk. Through 18 months' storage there were not appreciable losses of vitamin A in any of these products, the retention ranging from 90 to 100%.

Florida Warehouse Storage: Several of the canned foods stored in the Florida warehouse were lost and complete data on all the food items as originally planned are therefore not available. There were no remaining samples of tomato juice and

TABLE 5

Comparison of Retentions of Ascorbic Acid and Thiamine Following 12 and 18 Months' Storage at Controlled Temperatures and Florida Warehouse Storage

Product	Ascorbic Acid				Thiamine			
	70°	90°	100°	W	70°	90°	100°	W
	percent				*percent*			
Spinach								
12 Months	86	75	63	83	— — — — — — — — — —			
18 Months	83	64	50	74	— — — — — — — — — —			
Orange Juice								
12 Months	84	54	26	75	71	57	53	72
18 Months	Not available				Not available			
Green Beans								
12 Months	— — — — — — — — — —				Not available			
18 Months	— — — — — — — — — —				50	58	46	42
Peas								
12 Months	90	88	80	86	78	60	48	80
18 Months	78	72	66	86	83	56	35	74
Lima Beans								
12 Months	Not available				Not available			
18 Months	75	82	75	73	68	39	45	61

— — — — — — — — — — Dashes indicate food was not assayed for that vitamin.

apricots and portions of several other foods were also missing so that there were not sufficient samples for assay at both 12 and 18 months' storage. An inspection of the stability data for the stable nutrients shows that at Florida warehouse storage carotene, riboflavin, niacin, and vitamin A were as stable as at the controlled storage

temperatures where there was little or no temperature effect. Increases in riboflavin content were also typical in the warehouse storage samples and the slight decrease noted for the riboflavin content of Army Spread at 100° F. storage was not evident in the 12 months' warehouse storage sample. Eighteen month warehouse storage samples of this product were not available.

Retention of the labile nutrients, ascorbic acid and thiamine at warehouse storage following 12 and 18 months approximated the retention of these nutrients at 70° F. storage for the same periods of time. See Table 5. The yearly temperature fluctuations encountered in the Florida warehouse reported by Moschette *(15)* ranged from 54-91° F. with a yearly average of 77° F. These workers also found that the retentions of these heat-labile vitamins in warehouse stored samples correlated with the average yearly temperature occurring in the warehouse.

DISCUSSION

As stated, the cooperative high temperature storage study was undertaken primarily to furnish the Army the necessary information on the effects of elevated temperature storage on the nutrient content of canned foods. Several high temperature storage studies on canned foods had been conducted previously by the Army; but the results obtained could not be utilized with complete certainty until further checked on a large number of samples originating from the same source. The great fluctuation in nutrient content of canned fruits and vegetables from assay to assay on a ration storage study was always associated with a lack of uniformity in history (variety, soil and season grown in, processing treatments, etc.) of recurring samples in rations. It was felt that a study such as the one under discussion, containing large numbers of samples from a single lot of material, would minimize if not completely avoid sampling variations of the type occurring in the other investigations and thereby furnish reliable information on vitamin retention.

The results of the present study, though attaining the goal of setting vitamin retentions in stored canned foods, demonstrate the wide variation from can to can even in recurring samples from a single lot *(14)*. The lack of uniformity in nutrient content, especially for carotene, and to a lesser degree thiamine and ascorbic acid where variations were masked by storage losses, indicates the inadequacy of the 6 can composite sampling at each examination to overcome sampling differences in materials from a single lot. In order to ascertain the significance of the variations, the spread of values obtained for the more stable nutrients, carotene, riboflavin and niacin through the 18 months' storage period for the canned foods studied, was compared with the spread of these nutrients obtained for different packs of the same canned foods through a one to two year period *(9, 11, 17)*. It is seen in Table 6 that with the exception of spinach and apricots the range of carotene values for 2 years' packs is much wider than for samples originating from a single pack. The carotene range for spinach and apricots was approximately the same regardless of the type of origin. It is apparent therefore that although 6 can composite sampling minimizes the fluctuations due to natural variation and perhaps eliminates it in some instances, it is not sufficient for some of the foods studied. The spread of values for riboflavin in the canned fruits and vegetables throughout the 18 months' storage period also attained that present in the one year's packs for any one food. The riboflavin values did not fluctuate over the spread indicated but increased progressively on storage. In view of the number of studies reporting increases in riboflavin on storage, this spread of values may be considered an accompaniment of storage. The niacin values, unlike those of carotene, riboflavin, thiamine and ascorbic acid, showed a marked consistency through the 18 months' storage period and the range

TABLE 6

Comparison of Spread of Carotene, Riboflavin and Niacin Values From Single and Multiple Packs

Canned Food	(9) Carotene mg./100 gm	(17) Riboflavin mg./100 gm.	(11) Niacin mg./100 gm.
Apricots			
M. P.	0.93 - 1.74	.....	.....
S. P.	0.82 - 1.61	.....	.....
Green Beans			
M. P.	0.08 - 0.34	.....	.....
S. P.	0.11 - 0.14	.....	.....
Carrots			
M. P.	3.86 -11.35	.....	.....
S. P.	7.1 - 9.3	.....	.....
Yellow Corn			
M. P.	0.04 - 0.16	0.025-0.072	0.56-0.95
S. P.	0.026- 0.038	0.028-0.089	0.85-0.98
Peas			
M. P.	0.15 - 0.51	0.025-0.10	0.62-2.69
S. P.	0.20 - 0.29	0.048-0.114	1.01-1.27
Spinach			
M. P.	1.68 - 4.81	0.024-0.13	.....
S. P.	1.7 - 3.4	0.080-0.20	.....
Tomato Juice			
M. P.	0.16 - 0.96	0.009-0.046	0.41-0.97
S. P.	0.48 - 0.55	0.023-0.063	0.70-0.79
Lima Beans			
M. P.	.....	0.023-0.062	0.32-0.77
S. P.	.....	0.038-0.084	0.48-0.66

obtained for samples from a single lot was very narrow as compared to that for samples representing one year's packs.

On the whole the storage results are in agreement with those obtained previously *(1, 8, 15)*. Thiamine and ascorbic acid were the most susceptible to loss on storage, and carotene, riboflavin, and niacin were the least affected. Good retention of ascorbic acid, thiamine, riboflavin, and carotene was found by Moschette *(15)* in canned foods stored at 65° F. or below for periods of one year. Guerrant *(8)* also found excellent retention of these same vitamins under the same storage conditions with the exception of carotene which decreased by approximately 25% at 30° F. storage in tomato juice, lima beans and yellow corn. At increased storage temperatures (80° F. and above) these workers reported appreciable losses for thiamine and ascorbic acid in the canned foods tested, the greatest losses occurring at the higher storage temperatures. Guerrant also found increased losses in carotene content of the three foods studied and Moschette reported a 15% decrease in the carotene content of canned peaches following one year's storage at 80° F. In the current study, retention of nutrients was good at 70° F. storage with the exception of carotene in orange juice and green beans and thiamine and ascorbic acid in canned apricots and green beans where significant losses occurred following 18 months' storage at this temperature (20-40%). At the 90° and 100° F. storage, losses of thiamine and ascorbic acid, were increased. It is seen from the charts (Figures 1 and 4), that there is a linear relationship between the log percent retention of thiamine and ascorbic acid and the length of storage. The destruction of these two vitamins proceeds according to a first order reaction. Except for orange juice and green beans no appreciable decreases were noted in carotene content of these foods at any storage temperatures. The large variation in carotene content of recurring samples may have covered up

the larger carotene losses noted by Guerrant. The increase in riboflavin found in this study was not reported by either Guerrant or Moschette. In Guerrant's study the riboflavin content showed a slight decline at both storage temperatures (85 and 110° F.), that of the tomato juice being most adversely affected (60% retention after one year's storage at 110° F.). An increase in riboflavin content of dehydrated meat *(16)*, canned meats *(7)*, biscuits and cereals *(1)* stored at elevated temperatures have been reported both by the chemical and microbiological methods. Cooking and processing of foods have also resulted in increased riboflavin content *(2, 10, 20)*. However in none of these investigations did the magnitude of the increases approach that demonstrated in this study. The biological availability to rats or humans of the "apparent riboflavin" appearing on storage or cooking has as yet not been tested. An explanation of this phenomenon awaits such a determination.

Since the purpose of this study was to arrive at retention figures for the various nutrients in canned foods under the extremes of time and temperature storage conditions to which they might be subjected prior to consumption, the retention following 6 months' storage at 100° F. or 1 year at 90° F. was considered the criterion.[1] The data obtained indicated that the stability for the various nutrients in canned fruit and vegetables in general may be expressed as the following average percentages:

	6 Months at 100° F.	12 Months at 90° F.
	percent	*percent*
Thiamine	60	60
Riboflavin	100	100
Niacin	100	100
Ascorbic Acid	65	70
Carotene	100	100
Vitamin A and Carotene in Canned Dairy Products	100	100

It should be borne in mind that individual foods differ somewhat in their ability to retain nutrients, as for example, retention of ascorbic acid in tomato juice being superior to that in orange juice. However these differences are not of sufficient magnitude to warrant inclusion of separate retention figures. In view of the great variability possible in vitamin content of any one food found in the rations, it would be impractical to make further attempts at getting more accurate retention figures.

SUMMARY

1. A study of the stability of the various vitamins occurring in 13 representative canned foods used in Army rations including 4 canned dairy products, was made through an 18 months' storage period at 70, 90, and 100° F. Acceptability changes on storage were also followed. The results showed that:

a. There was an 85 to 100% retention of vitamin A and carotene in the canned dairy products studied regardless of storage time and temperature. Riboflavin was retained 90 to 100% in canned dairy products except Army Spread in which the retention was approximately 70% after 18 months' storage at 100° F.

b. Carotene and niacin content of the canned foods was maintained 90 to 100% at all storage temperatures except in the case of yellow corn, green beans and orange juice in which the carotene content dropped approximately 15-30% below this level.

c. The riboflavin content of the canned foods except dairy products showed tremendous increases on storage (25-200%) at all three temperatures. There was no apparent temperature relationship.

[1] These test conditions are an accelerated representation of actual field conditions, since actual storage temperatures are somewhat lower in most cases but storage times are normally longer.

d. Thiamine and ascorbic acid decreased on storage; the degree of decrease being a function of storage time and temperature. Following 6 months' storage at 100° F., the average retention for thiamine and ascorbic acid was 60 and 65% respectively.

ACKNOWLEDGMENT

The authors are indebted to the Research Departments of the American Can Company, Continental Can Company and the National Canners' Association for their cooperation in planning the study, in the collection of samples, in providing storage facilities, and for their assistance with the analytical work. Appreciation is also expressed to Dr. Elvehjem for providing the folic acid storage data, to Miss Ruth Epstein of the QMF&CI for the statistical calculations and to the following individuals for their valuable suggestions: Drs. Clifcorn, Feaster, Ives, Fevold, and Mr. Heberlein.

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EFFECT OF DIFFERENT PROCESSING PROCEDURES ON VENTING AND LOSS OF LIQUID FROM HOME CANNING JARS [a, b]

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Objective tests demonstrate the conditions for most effectively exhausting residual air and reducing loss of liquid in the home-canning of foods thus contributing to the retention of desirable qualities of color, flavor, and vitamin content.

Home canners frequently complain of an excessive loss of liquid from jars during processing, particularly in the case of products processed in a pressure canner. In order to obtain additional information on factors which may contribute to this loss of liquid, an investigation has been carried on to observe the influence of pressure canner operation and type of jar closure as causative agents. This problem was studied several years ago by Fellers, Levine and Maclinn *(1)* using bail-type ("E-Z Seal") all glass jars with wire bails. They reported that fully sealing all glass jars prior to processing seemed to answer the problems of reducing liquid losses and improving the general appearance of the product. Fully sealing the jars, releasing pressure slowly, and leaving a large headspace decreased the loss of liquid in pressure processed jars. As a result of a two-year investigation of the vacuums and internal pressure of all-glass canning jars during heat processing and of practical cooperative experience involving over 16,500 all-glass jars these authors recommended sealing the jar by tightening the wire bail (full seal) prior to processing.

With home canned foods, particularly fruits, oxygen or air entrapped within the container can be an important cause of surface darkening, off flavors, and loss in vitamin content. The venting of home canning jars during processing is effective in permitting the release of air that is entrapped in the headspace or product. The effect of type of closure and method of processing on the effectiveness of venting in removing air from the jars has also been observed in the present investigation.

Fellers, Levine, and Maclinn *(1)* presented data which showed that, partially sealed bail-type jars, when processed for up to 20 minutes in a boiling water bath, had a considerably higher vacuum than did fully sealed jars. Water was used as a test medium in the jars. When processed in a pressure canner at 10 pounds steam pressure both the partially sealed and the fully sealed jars had good vacuums. The vacuums in the fully sealed jars were only slightly lower than those of the partially sealed containers.

EXPERIMENTAL

Effect of Pressure Canner Operation on Loss of Liquid from Jars. Pint jars filled with previously boiled tap water were processed in a pressure canner operated by four different procedures in order to observe the effect of method of operation on loss of liquid. In all cases the jars were filled with water at 70° F. (21° C.), leaving a 50 cc. headspace (approximately one half inch). The types of jars and seals studied were: (1) Bail-type jar fully sealed prior to process; (2) Bail-type jar partially sealed; (3) Two-piece metal lid closure fully sealed; (4) Three-piece glass lid partially sealed prior to processing, the band being turned back one-half inch in one series of tests and one-quarter inch in another series. The zinc P/L mason cap was turned back one-quarter inch. After processing the seals were completed when

[a] Contribution No. 649 Massachusetts Agricultural Experiment Station.
[b] Acknowledgment is due the Hazel-Atlas Glass Company, Wheeling, West Virginia for their interest and cooperation in carrying out this investigation.

necessary and the jars were allowed to cool overnight. The following day the loss of liquid that occurred during processing was measured by filling the jars to overflowing with water and determining the difference in volume between the original headspace and that after processing. In each test nine pint jars were processed in a seven quart capacity aluminum pressure canner.

As standard practice in each test one inch of water was placed in the pressure canner and the jars were added. After it was sealed the canner was heated and allowed to vent for 10 minutes before closing the pet cock and bringing the steam pressure up to 10 pounds, 240° F. (116° C.). The jars were processed for 45 minutes at this temperature. The methods of pressure canner operation employed were as follows:

1. A steady 10 pound pressure was maintained throughout the process. The canner was cooled very rapidly by opening the pet cock at the end of the process. The canner was then opened immediately and the jars removed.
2. Same as (1) except that at end of process the canner was moved from the burner and the pressure was allowed to drop normally to 0 pounds. The canner was then opened and the jars were removed.
3. Same as (2) except that the canner was allowed to cool an additional 15 minutes, after the pressure had dropped to zero, before it was opened and the jars removed.
4. During the process the pressure was maintained at 10 pounds for the first 10 minutes. The heat was then cut off and the pressure was allowed to drop to eight pounds where it was maintained for five minutes. The pressure was then raised to 12 pounds and held at that point for 10 minutes after which it was dropped back to 10 pounds for the remainder of the process. The canner was allowed to cool slowly to zero and the jars were then removed. This method was used to demonstrate the effect of fluctuating pressure during processing.

The results of these tests, which are averages of several runs in each case, are presented in Table 1. It may seem that the relative tightness of the jar seal dur-

TABLE 1

Effect of Method of Pressure Canner Operation and Type of Jar Closure on Loss of Liquid from Jars During Processing

Method of pressure canner operation	Loss of Liquid					
	Bail Type		Two-piece metal lid, full seal	Three-piece glass lid,[1] partial seal	Three-piece glass lid,[2] partial seal	Zinc P/L cap partial seal
	Full seal	Partial seal				
	ml.	ml.	ml.	ml.	ml.	ml.
1. Pressure dropped rapidly after process	7.2	22.3	3.4	2.6	35.4	26.6
2. Slow cool. Jars removed when pressure reached 0 pounds	5.0	11.1	1.0	0.5	10.2	14.7
3. Slow cool. Jars removed 15 minute after pressure reached 0 pounds	4.5	7.9	0.5	0.0	7.6	15.2
4. Pressure fluctuated during processing, slow cool	8.8	12.5	2.2	4.2	20.0	18.6

[1] Metal band turned back ¼ inch to make partial seal.
[2] Metal band turned back ½ inch to make partial seal.

ing processing has a definite effect on the tendency of the jar to lose liquid during processing in a pressure canner.

Closures of a type that require a partial seal showed a greater loss of liquid than those that are fully sealed during processing. Faulty operation of a pressure canner in a manner that permitted the pressure to fluctuate during processing or too rapid cooling (such as opening the pet cock as soon as the process was completed) also caused a greater loss of liquid.

Further tests were made in order to observe the effect of full and partial sealing, processing in slow and rapid cooling pressure canners and fatigue of wire bails on the loss of liquid from bail-type home canning jars during processing and cooling. Seven new and unused quart jars were used in each test. The jars were filled to overflowing with water at room temperature, 70° F. (21° C.), and 50 ml. of the water was removed with a pipette, to provide a 50 cc. headspace (approximately one-half inch) below the overflow point. A seven quart capacity heavy aluminum pressure canner was used as a typical slow cooling canner and a steel pressure canner of similar size was used as a rapid cooling canner. Seven quart jars were processed at a time. In all cases the jars were processed for 60 minutes at 10 pounds steam pressure, 240° F. (116° C.). Care was taken to maintain uniform conditions in all tests so that the results would be comparable. After processing the jars were allowed to cool overnight and the headspace was measured. The increase in headspace volume after processing was taken as the loss of liquid.

Tests were made on several consecutive days with the same set of jars under a given test condition to observe whether or not the wire bail showed fatigue or became loose during use and thus permitted a greater loss of liquid during processing and cooling.

As may be seen in Table 2, the pressure canner that cooled rapidly caused a greater loss of liquid in the jars than did the slow cooling canner. Likewise, as shown previously, the liquid was better retained in the jars which were fully sealed prior to processing. During usage up to six or seven consecutive times the wire bails on the bail-type jars did not show any marked tendency to loosen and allow a significantly greater loss of liquid.

EFFECT OF PROCESSING METHOD ON VENTING CHARACTERISTICS OF HOME CANNING JARS

Pint jars were filled with (1) boiling water and (2) previously boiled water at 70° F. (21° C.), leaving headspaces of 25, 50 (approximately one-half inch), 75, and 100 cc. The jars were then sealed and processed. The processed jars were allowed to cool overnight and the volume of residual air in the jars was measured by the water displacement method, Maclinn and Fellers *(2)*.

Five different variations of jar closures were investigated:

1. Bail-type jar, partial seal
2. Bail-type jar, full seal
3. Three-piece glass lid, partial seal, band turned back one-half inch
4. Two-piece metal lid, full seal
5. Zinc P/L Mason Cap, partial seal, cap turned back one quarter inch

Groups of the different types of jars with the variations in headspace and filling temperature were processed under different conditions as follows:

1. Boiling water bath—15 minutes
2. Boiling water bath—30 minutes
3. Boiling water bath—120 minutes

TABLE 2

Loss of Liquid from Quart Bail-Type Jars as Influenced by Full and Partial Seal During Processing, Slow and Fast Cooling Pressure Canners, and Fatigue of Wire Bail

Type of Canner Run No.	Jar Seal	Average loss of liquid	Pressure Canner cooling time
		ml.	min.
Slow cooling			
1	partial	14.4	20
2	partial	21.6	22
3	partial	18.6	30
4	partial	16.1	22
5	partial	20.0	30
6	partial	20.3	24
7	partial	33.3	34
average	partial	20.6	26
Slow cooling			
1	full	6.3	20
2	full	2.6	28
3	full	4.1	25
4	full	3.0	32
5	full	4.9	21
6	full	5.7	25
7	full	6.1	23
average	full	4.7	25
Rapid cooling			
1	partial	21.6	16
2	partial	41.4	11
3	partial	40.9	10
4	partial	36.3	9
5	partial	32.6	11
6	partial	29.3	16
average	partial	33.6	12.2
Rapid cooling			
1	full	7.7	13
2	full	7.6	10
3	full	7.0	11
4	full	8.0	11
5	full	7.5	12
average	full	7.5	11.4

4. Pressure canner—15 minutes at 0-1 pound steam pressure
5. Pressure canner—30 minutes at 0-1 pound steam pressure
6. Pressure canner—1 minute at 5 pounds steam pressure
7. Pressure canner—5 minutes at 5 pounds steam pressure
8. Pressure canner—1 minute at 10 pounds steam pressure
9. Pressure canner—60 minutes at 10 pounds steam pressure

From nine to 27 jars were processed in from one to three groups under each variable and the results presented represent averages of all the jars in each variable. In all of these tests usual recommended home canning procedures were followed in order to obtain information on the effect of the different factors under practical conditions. New closures, and new jars in the case of bail-type jars, were used in all tests. The total number of individual jars and closures tested amounted to approximately 6500.

Space does not permit a detailed presentation of all the data obtained. Table 3 shows the results obtained with jars filled with boiling water allowing a 50 cc. headspace. The venting performance of the various jar closures under the different

processing conditions is typical of that found in all of the tests that were made. The results are reported in terms of volume of residual air rather than actual vacuum because we are primarily interested in the amount of air left in the jars which might be available to cause deterioration of the product during storage.

As may be seen from Table 3 there was a definitely better exhaust of air from the partially sealed jars than from the fully sealed jars during short processes such as are used for fruits. With longer process times, or during processing at higher temperatures in a pressure canner, all of the different types of jars were more completely vented, with the result that process times for vegetables in a pressure canner can be expected to provide a very complete elimination of air from any of the common types of home canning jar closures.

TABLE 3

Venting Characteristics of Home Canning Jar Closures Under Different Processing Conditions

Processing method	Volume of residual air				
	Bail Type		Three-piece glass lid, partial seal	Two-piece metal lid, full seal	Zinc P/L Mason cap, partial seal
	Partial seal	Full seal			
	cc.	cc.	cc.	cc.	cc.
Boiling water bath					
15 minutes	6.7	20.5	6.0	9.8	7.5
30 minutes	5.5	16.2	4.0	12.0	5.0
120 minutes	4.7	18.3	1.2	9.4	1.9
Pressure canner					
0-1 lb. steam, 15 minutes	4.0	16.5	4.0	9.0	4.6
0-1 lb. steam, 30 minutes	6.2	15.5	2.0	8.0	2.8
5 lb. steam, 1 minute	5.0	11.2	3.0	10.5	2.6
5 lb. steam, 5 minutes	1.2	13.4	2.3	9.2	4.1
10 lb. steam, 1 minute	2.2	5.0	0.8	11.0	3.7
10 lb. steam, 60 minutes	1.0	2.2	1.2	3.2	5.2

With short time processes in a boiling water bath, jars which were filled hot contained less residual air than those which were cold filled. The volume of residual air increased with an increase in headspace volume, and in all cases fully sealed jars contained more residual air than did those which were partially sealed. As the time and temperature of the process increased the effects of headspace, fill temperature and type of closure diminished until with a one hour process of 240° F. (116° C.), all types of jars and closures under all conditions allowed a very complete exhaust of air from the jars.

SUMMARY

The data obtained in this study serve to point out certain factors which have a direct bearing on the loss of liquid from home canning jars during processing in a pressure canner and how it may be controlled. A loose or partial seal on the jar, fluctuation of the pressure during processing, and rapid cooling of the pressure canner after processing are factors which contribute to a loss of liquid. Even with partially sealed jars good pressure canner operation can contribute markedly towards preventing an excessive loss of liquid.

To keep the loss of liquid at a minimum the following procedure of operating the pressure canner is recommended: Maintain a constant even pressure during processing. At the end of the process time turn off the heat or slide pressure canner away from the source of heat on the stove. Allow natural cooling and do not open vent until zero pressure is reached. Open pet cock or vent as soon as zero pressure is reached, then allow five minutes to elapse before opening the canner. In any

event do not open canner less than 20 to 25 minutes after the heat is shut off at the end of the process period. If the canner cools more rapidly than this it may be left on a warmer portion of the stove or on very low heat during part of the cooling period.

For fruits and other acid foods, which are usually given a short process in a boiling water bath, a partial seal on the jars permits a more effective exhaust of residual air. If a full seal is used, a hot fill and small headspace (one quarter inch) are effective in reducing the amount of residual air left in the jars after processing. With such products a loss of liquid from partially sealed jars does not present a serious problem.

On the other hand, with low acid foods which are processed in a pressure canner at 10 pounds steam pressure the use of a full seal to prevent an excessive loss of liquid will still provide satisfactory venting and permit most of the entrapped air to be exhausted from the jars during processing.

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A TECHNIQUE FOR STUDYING RESISTANCE OF BACTERIAL SPORES TO TEMPERATURES IN THE HIGHER RANGE*

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This paper presents details of a new technique, giving greater accuracy in less time than other methods, for determination of thermal resistance of bacterial spores in the range of 215° F. (101.6°C.) to 270°F. (132.2°C.). The value of data obtained by the new technique in the calculation of thermal processes for foods is discussed.

Several methods have been employed in studies designed to obtain information, concerning the thermal resistance of bacteria, upon which to base calculation and evaluation of thermal processes for foods. In general, the methods which have been described in literature for studying thermal resistance of bacteria to temperatures above 212° F. (100° C.) may be roughly classified as follows:

1. Thermal death time (TDT) tube method *(5)*
2. Thermal death time (TDT) can method *(1)*
3. Rate of destruction method *(10)*

Various modifications of each of these methods are in use today for studying thermal resistance of bacteria to temperatures as high as 250° F. (121.1° C.); however, it is common knowledge that none of the methods is readily adaptable for studying thermal resistance of bacteria to temperatures above 250° F. (121.1° C.). In fact, there is reason to believe that the TDT tube and can methods are not adaptable for studying thermal resistance of bacteria in many foods to temperatures above about 240° F. (115.56° C.) because of the lag in temperature rise at points relatively remote from the surface of the food mass in the TDT container. Methods have been proposed *(8)* for arriving at corrections to account for the temperature lag in TDT tubes and cans; however, because corrections arrived at in this way apply to lethality of heat treatment at only one point (point of greatest temperature lag) in the container, there is good reason to doubt the validity of such corrections [see discussion relating to consideration of temperature distribution in calculating thermal processes for foods *(9)*].

The lack of knowledge as to the exact nature of thermal death time curves for bacterial spores subjected to temperatures above 240° F. (115.56° C.) has been largely due to the inadequacy of methods heretofore available for studying thermal resistance of the spores to the higher temperatures. It has been well established that thermal death time curves for spores subjected to temperatures in the range of 212° F. (100° C.) to 240° F. (115.56° C.) are in general straight lines when plotted on semi-logarithmic coordinates, temperature being plotted on the linear scale and time on the log scale. Various attempts to establish the nature of the curves for spores subjected to temperatures above 240° F. (115.56° C) have contributed data which in several instances have been interpreted as indicating that the curves for the higher temperatures are not straight. As a result, opinion is now divided as to whether the straight line curves obtained when lower temperatures are employed may be extrapolated as straight lines to ascertain resistance values for temperatures in the higher range. Since mathematical methods of process calcula-

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tion *(2, 3)* are based on the assumption that semi-logarithmic thermal death time curves are straight, establishment of the exact nature of the curves for spores subjected to the higher temperatures is imperative if these methods are to be applied with confidence in calculating thermal processes employing such temperatures. In these methods of calculation the term "z" is universally employed to represent the slope of the straight line semi-logarithmic thermal death time curve. The value of z is taken as the number of degrees Fahrenheit required for the curve to traverse one logarithmic cycle. Obviously, these methods would be inapplicable without considerable modification if semi-logarithmic thermal death time curves were not straight. Moreover, whatever the nature of the curves, the accuracy of process determination, other things being equal, depends upon the accuracy of values upon which the curves are based regardless of the method of process calculation employed. Methods heretofore available have not been adequate for obtaining accurate thermal resistance data for spores subjected to temperatures in the higher range, above 240° F. (115.56° C.)

With the growing interest in the use of higher temperatures for processing a variety of food products, the need for methods for determining accurately the thermal resistance of spores to these higher temperatures is becoming increasingly urgent. For this reason the present study was undertaken. The method developed is now being successfully employed in this laboratory for measuring the thermal resistance of spores to temperatures as high as 270° F. (132.22° C.).

DEVELOPMENTAL CONSIDERATIONS

Requirements of a satisfactory method of heating and cooling inoculated samples of food were considered to be somewhat as follows:

1. Permit of maintaining food at any given temperature between 220° F. (194.44° C.) and 270° F. (132.22° F.).
2. Permit of using heating times, in the range of five seconds upward, which could be accurately measured and repeated.
3. Accomplish virtually instantaneous heating of the food sample to the desired temperature.
4. Accomplish virtually instantaneous cooling of the food sample, after heating, to temperatures the lethality of which would be negligible compared with the lethality of the temperature being employed to determine bacterial resistance.
5. Yield data from which rates of destruction of bacteria, subjected to any lethal temperature up to 270° F. (132.22° C.) could be ascertained.
6. Simple and inexpensive to operate compared with methods heretofore available for studying thermal resistance of bacteria in foods.

Heating and Cooling. After consideration of the characteristics of different heating media, steam was chosen as the most suitable in all respects. High frequency heating was carefully considered but not tried because it was anticipated that, in high frequency heating, accurate control of temperatures of foods with different heating characteristics would be difficult.

To accomplish virtually instantaneous heating of a food sample in steam two things appeared to be imperative, namely, the sample would have to be small and it would have to be introduced into a chamber containing live steam at the pressure corresponding to the temperature to be employed in the thermal resistance determination. By use of mathematical methods presented by Olson and Schultz *(6)* it was estimated that the temperature of a layer of food 0.5 mm. in depth would, if the food were placed in steam at 270° F. (132.22° C.), increase from 175° F. (79.44° C.) to 269.7° F. (132.06° C.) in one second—the temperature referred to being that of the food at a point in the layer most remote from the surfaces. Since it was anticipated that heating times at 270° F. (132.22° C.) no shorter than about

four or five seconds would be used, the possibility of attaining sufficiently rapid heating of the sample to give reliable results seemed promising.

Several possibilities for virtually instantaneous cooling were considered. The most feasible seemed to be cooling by evaporation from the sample. It was reasoned that if the food were heated in an open container very rapid cooling could be accomplished by a rapid pressure release at the end of process when the food would be at the temperature the effect of which was being measured. Since the layer of food was not to exceed 0.5 mm. in thickness it was believed that evaporation would occur at the food surface, when the pressure was released, without loss of food due to ebullition. This belief was confirmed by experimentation and the method of cooling incorporated in the procedures to be described.

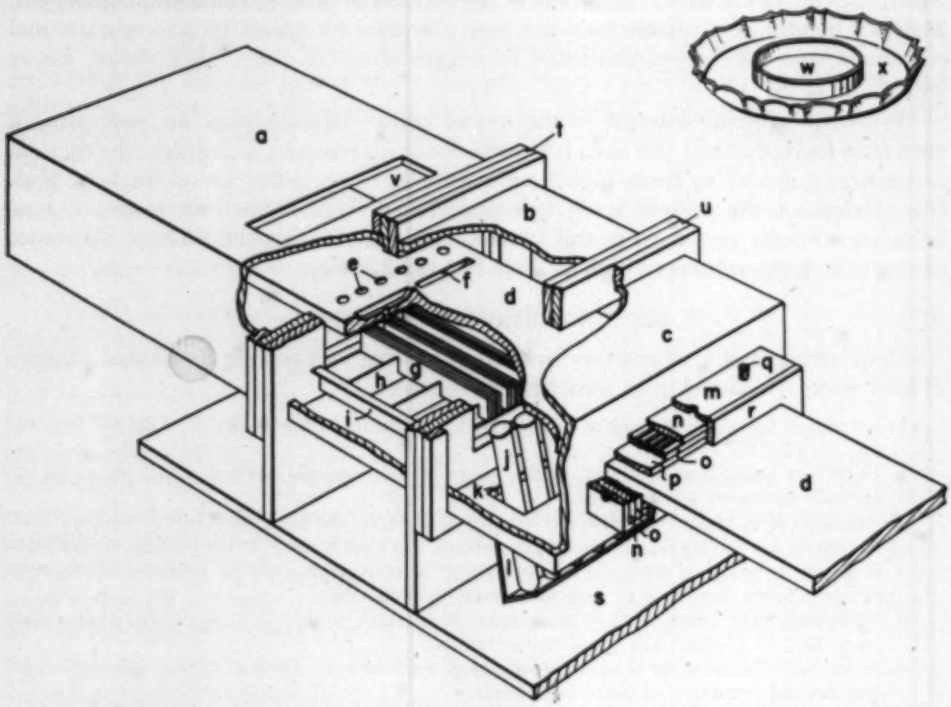

FIG. 1. Perspective drawing showing a longitudinal vertical cut-out section of thermoresistometer (not drawn to scale).

Design of Heat Exchanger. Figure 1 is a perspective drawing showing a longitudinal vertical cut-out section of the heat exchanger and the mechanism for introducing and withdrawing the food samples. A number of the parts have been lettered to facilitate description. The three steam chambers *(a, b,* and *c)* are so arranged that, when closed by covers at openings *v* and *k* in chambers *a* and *c,* the chambers and all mechanisms therein may be sterilized by live steam at pressures up to 50 pounds (gauge). Chamber *a* is 13½ inches long by 9 inches wide by 4¼ inches deep, chamber *b* is 4¾ x 9 x 4¼ inches, and chamber *c* is 4 x 9 x 4¼ inches (outside dimensions). The ends of chamber *b* (center chamber) as well as forming partition plates, extend downward as indicated to serve as supports (legs joined to the base platform *s*). Secondary partition plates *t* and *u* are also inserted between chambers *a* and *b* and chambers *b* and *c* respectively. These two partition plates carry bearing housings (not shown in drawing) identical to the bearing housing

m-r shown attached to chamber *c*. Slots were cut in the partition plates and the one end plate of chamber *c* to allow insertion of carrier plate *d*. These slots (approximately ¼ x 5-9/16 inches) are centered vertically and horizontally so as to allow insertion of plate *d* in the position indicated.

The chambers and partition plates are held in the position indicated by bolts through the chamber flanges and partition plates. Three flat seal bearing arrangements (in bearing housings mentioned above) around plate *d*, in position indicated, allow steam pressures to be maintained in any one chamber individually without appreciable leakage of steam from one chamber to another or to the outside. Each seal bearing arrangement consists of two bronze seal plates *p*. one above and one below plate *d;* packing *o*, above and below and at edges of carrier plate *d;* compression plates *n;* and, compression screws *q*. Plate *r* forming part of the bearing housing is removable thus permitting the bearings to be assembled with plate *d* in position.

Plate d is a stainless steel plate 15/64 inch thick, 5½ inches wide and approximately 39¼ inches long. This plate was carefully surface ground so that a nearly perfect seal could be obtained with each of the three flat seal bearing arrangements mentioned above. The contacting surfaces of each bronze seal plate *(p)* and the carrier plate *d* were carefully lapped to obtain near perfect contact of the seal plates with the carrier plate. The six 11/16 inch diameter holes *(e)* in plate *d* are about 10¼ inches from the one end of the plate. The narrow slots *(f)* in plate *d* are traps for removing condensate. When plate *d* which is propelled by turning the crank of the drive mechanism (see Figure 2) is in such a position that the holes are directly below opening *v* in chamber *a*, the holes are closed below by a small metal platform, the top surface of which is level with the top surface of the lower seal plate in the bearing between chambers *a* and *b*. The top surface of this bearing plate is level with the top edges of the grill plates *(g)*, with the top surface of the lower seal plate in the bearing between chambers *b* and *c*, and with a second small metal platform extending into chamber *c* from the housing of this bearing. The grill work *(g, h, i)* is mounted on four compression springs so that the grill plates *(g)* are kept in contact with the lower surface of plate *d* without impeding the movement of plate *d*. This entire arrangement permits of food containers (small cups) placed in the holes *(e,* plate *d)* to be drawn into chamber *b* and then from chamber *b* into chamber *c*. Just after the food cups enter chamber *c* they are drawn over a narrow platform from the edge of which they drop straight downward from the holes *(e)* in plate *d* into tubes of culture media *(j)* extending into chamber *c* through the opening *k*. The tubes (22 x 175 mm.) are held in position by a simple rack arrangement *(1)*.

CONTROL AND OPERATION

Figure 2 is a photograph of the "thermoresistometer" as installed in this laboratory. The large horizontal tank (approx. 12 inches in diameter and 50 inches long) inside the wall cabinet is a steam reservoir to facilitate more accurate control of steam pressures in the thermal resistance chamber (*b*, Figure 1). Steam pressure in the reservoir is controlled by a Taylor pressure controller. Each of the three chambers of the heat exchanger is connected to the steam reservoir by a ⅜ inch copper tube provided with a shut-off valve and each is provided with a separate exhaust line, thus permitting of the same pressure being maintained in any one, or all, of the chambers as is maintained in the reservoir and permitting of each chamber being pressurized or exhausted independently of the other two. With this arrangement the temperature of the center chamber (provided with condensate trap in exhaust line) may be controlled to within 0.3° F. of any desired temperature.

Accurate control of pressures in chambers *a* and *c* is not imperative since these chambers are pressurized only for the purpose of pre-sterilization of the system.

Process time is accurately measured by an electric time clock which is started and stopped by two micro switches. Just as the food sample enters the pressurized chamber (*b*, Figure 1), a microswitch, operated by a cam fastened to the bottom surface of the carrier plate *d*, starts the clock. At the end of any desired process time, steam pressure in the heating chamber is released by simultaneously closing a valve in the steam supply line and opening a valve in the exhaust line. The lever

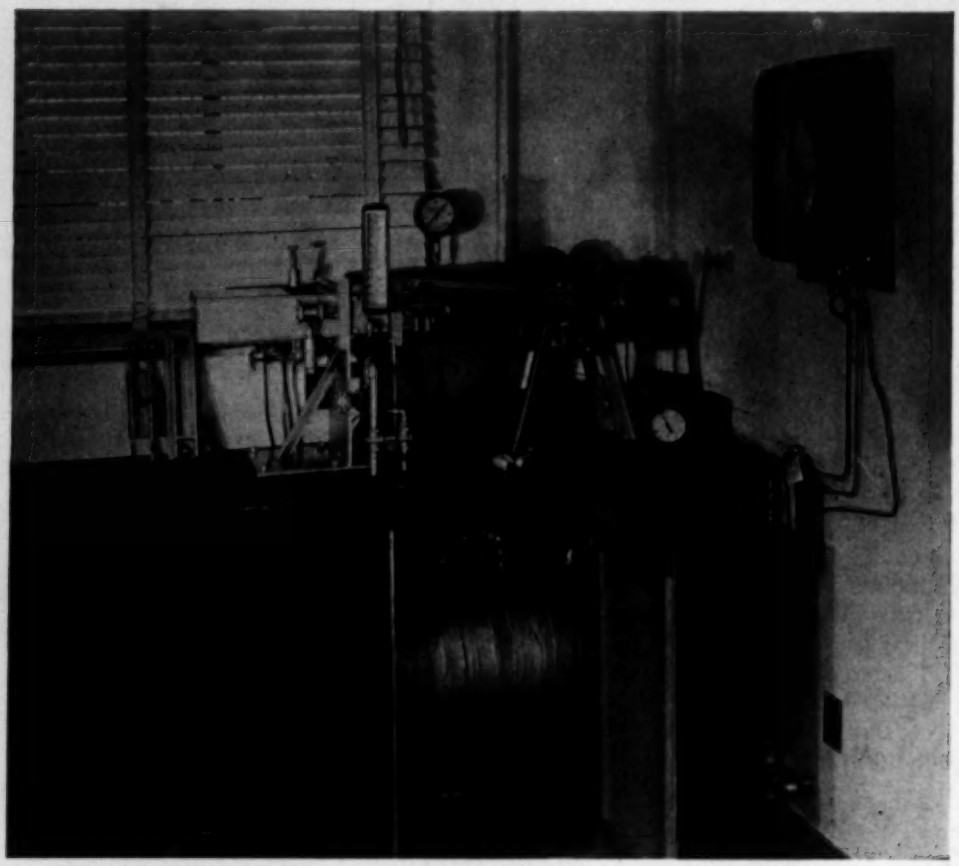

FIG. 2. Photograph of thermoresistometer as installed in this laboratory.

which operates these valves also operates a microswitch which stops the clock the instant the pressure in the chamber starts to fall. By this procedure heating times may be readily reproduced with an accuracy of plus or minus 0.001 minute.

The inoculated food samples are carried in small metal cups (*w*, Figure 1) which are approximately 7 mm. in diameter and 1.5 mm. deep. The cups are made of 0.011 inch thick tin plate. In preparing the inoculated food samples, 0.01 ml. of spore suspension (appropriate dilution of whole culture in distilled water) and approximately 0.01 ml. of food are placed in each cup, mixed with a sharpened inoculating needle, and spread uniformly in the cup. This gives a sample thickness of approximately 0.5 mm. (actually the thickness never exceeds 0.5 mm. because the sample spreads on the wall of the cup as well as on the bottom). It should be noted that

with the foods studied to date, the approximate 100 percent dilution of the food by spore suspension has been shown to have no detectable influence on spore resistance. This and other factors will be discussed in a later publication dealing with the microbiological techniques employed. Suffice it to say here that the thickness of the food sample in the cup is purposely kept to 0.5 mm. or less.

The small cup of inoculated food is placed in a larger aluminum boat with fluted edges (*x*, Figure 1). The aluminum boat is made from 0.003 inch thick aluminum sheet. It is shaped from an 11/16 inch circle by means of a simple manually operated die. The boat functioning as a carrier of the sample cup during processing prevents direct contact between the sample cup and the heavy metal carrier plate (*d*, Figure 1), thus facilitating more rapid heating of the sample. The fluted edges minimize contact with the carrier plate as well as allowing free circulation of steam in the region of the food sample.

Prior to a thermal resistance determination, all three chambers of the resistometer are sterilized. After sterilization, chambers *a* and *c* are exhausted. The covers are removed from openings *k* and *v*, and the pressure in chamber *b* is adjusted to give the desired operating temperature. An aluminum boat is then placed in each of the six holes (*e*, Figure 1) when the carrier plate is in such a position that the holes are directly below opening *v* in chamber *a*. A cup carrying a food sample is placed in each boat, and the samples are then drawn into chamber *b* where they are instantaneously subjected to the operating temperature. At the end of any prescribed time chamber *b* is exhausted and the heated samples are drawn into chamber *c* where they (boats, cups and samples) fall into tubes of culture media placed as indicated in Figure 1. The cultures of heated spores thus obtained are covered with a layer of sterile vaseline-paraffin mixture and incubated at the appropriate temperature. (The usual precautions are observed in handling the tubes of culture media to avoid contamination during transfer of the tubes to and from the resistometer).

DETERMINATION OF HEATING AND COOLING RATES

Because of the very rapid heating and cooling of the small samples of food, heating and cooling rates could not be accurately determined experimentally by the usual methods. However, by employing mathematical methods published by Olson and Schultz *(6)*, it was possible to arrive at curves representing rise and fall of temperature of samples during heating and cooling. Each food sample when processed is in the form of a layer, the thickness of which does not exceed 0.5 mm. Since the diameter of the circular sample is 7 mm., it may be assumed to heat as an infinite slab of food. The temperature at a point in a slab most remote from all surfaces after heating time t is given by the following formula:

$$U = S\,(K\,t/a^2) = S\,(\theta) = \frac{4}{\pi}\,(e^{-\pi^2\theta} - 1/3\,e^{-9\pi^2\theta} + 1/5\,e^{-25\pi^2\theta} \ldots\ldots) \text{ where:}$$

U = temperature on "theoretical" scale, related to T by,

$U = (T_1 - T)/(T_1 - T_o)$ where:

T = temperature on "practical" scale (Fahrenheit, Centigrade, etc.)

T_1 = temperature of heating or cooling medium.

T_o = initial temperature of object to be heated.

K = thermal diffusivity of object to be heated.

t = time (of heating or cooling).

a = thickness of slab.

Olson and Schultz *(6)* tabulated values for the function $S(\theta)$ corresponding to values of θ between 0.001 and 0.200. For values of θ greater than 0.200, they give the following equation for calculating $S(\theta)$.

$$S(\theta) = 1.27324 \log^{-1} (-4.28631\theta).$$

By means of values obtained from calculations using these formulae the curves in Figure 3 were established as representing temperatures existent at the geometric

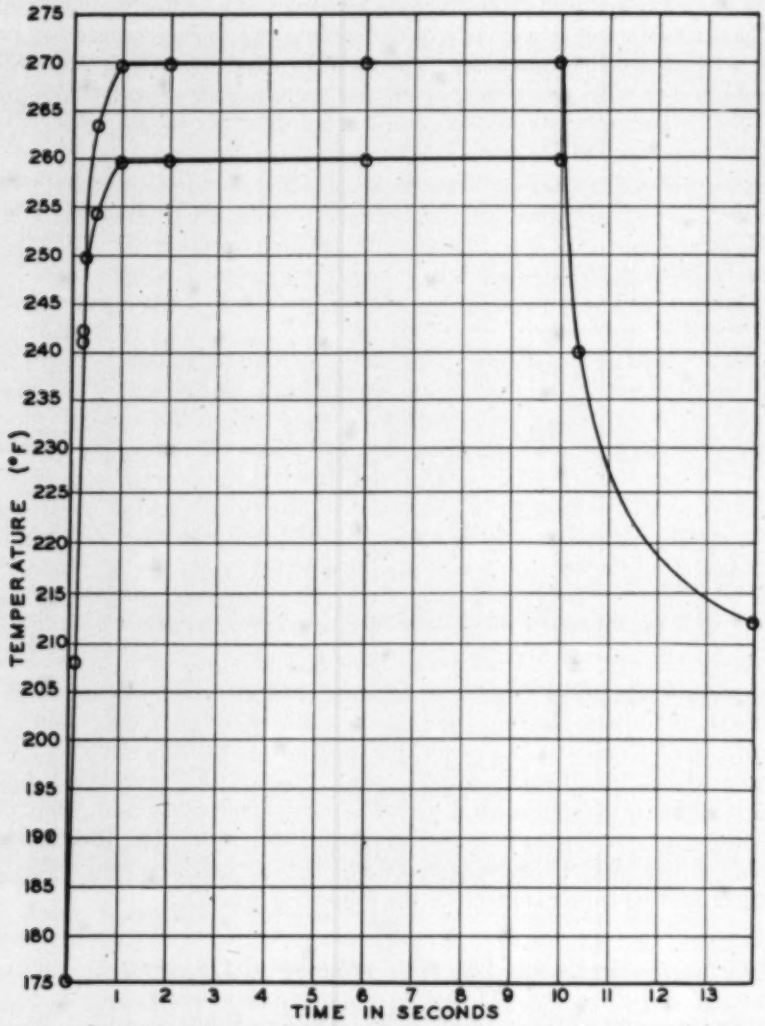

FIG. 3. Calculated heating and cooling curves representing temperatures existent in food sample, during thermal process, at point most remote from surface.

center of the food samples during ten second processes at 260° F. (126.67° C.) and 270° F. (132.22° C.) respectively. The calculations were made using the following values to represent terms in the above equations.

$K = 0.0015$ cm²/second (an approximate value).
$a = 0.05$ cm.
$T_1 = 270°$ F. (132.22° C.).
$T_o = 175°$ F. (79.44° C.).

In order to determine whether the metal cups would appreciably influence heating of the samples, calculations were made to determine the time required, after the samples entered the processing chamber, for the surface of the metal next to the food to reach virtually the temperature of the steam. It was established that in less than 0.04 second after samples entered the processing chamber at 270° F. (132.22° C.) the temperature of the metal surface next to the food would exceed 269.97° F. (132.20° C.). On this basis it was concluded that the metal cups would not appreciably influence heating of the food samples and that the heating and cooling curves established by calculation were sufficiently accurate for estimating errors due to heating and cooling lags if no corrections to account for such lags were applied, that is, if uniform heating of the samples to process temperature and uniform cooling of the samples from process temperature to a relatively non-lethal temperature were assumed to occur instantaneously.

If corrections computed according to the method of process evaluation *(2, 3)* which considers only the point of greatest temperature lag in the food mass were applied, they would amount to seven percent or less of the heating time at 270° F. (132.22° C.) for a process having a lethality value equivalent to one minute at 250° F. (121.1° C.), 3.5 percent or less of the heating time for a process having a lethality value equivalent to two minutes at 250° F. (121.1° C.) and correspondingly lower percentages of the heating times for processes with higher lethality values. For a process at 260° F. (126.67° C.) equivalent to one minute at 250° F. (121.1° C.) the correction would amount to two percent or less of the heating time; the correction would be correspondingly less for longer heating times. Corrections computed by this method are somewhat greater than they should be because the method considers only the point of greatest temperature lag in the food mass.

Though exact corrections would be very difficult to ascertain, it may be safely assumed that the maximum error in any heating time at any processing temperature up to 270° F. (132.22° C.), if corrections are not applied for temperature lag and if the thickness of the food layer does not exceed 0.5 mm., would not be greater than 0.3 second. Since the food samples are within 0.3° F. of steam temperature for at least 9 seconds of a 10 second process and for a greater percentage of the time for all longer processes, it is felt that in most cases no correction of the heating times would be necessary in order to obtain thermal resistance results of acceptable accuracy.

TREATMENT OF DATA

Death of bacteria as the result of the application of "moist" heat is essentially logarithmic in order *(7)*. For bacteria subjected to any given lethal temperature, if the number of surviving cells after varying intervals of time are plotted on the logarithmic scale against time of heating on the linear scale, a straight line curve will generally be obtained. The term "Z" (Zeta) is employed to represent the slope of the rate of destruction curve *(4, 9)*, the value of Z being taken as the number of minutes required for the rate of destruction curve to traverse one logarithmic cyle. Then as pointed out by Stumbo *(9)*:

$U = Z\ (\log a - \log b)$ where,

U = time (minutes) of heating.

a = initial number of cells (or spores).

b = number of cells remaining viable after heating time U.

then,

$$Z = \frac{U}{\log a - \log b}$$

This latter equation permits of calculating values of Z from data obtained by use of the thermoresistometer. Data obtained from a study of the thermal resistance of spores of the putrefactive anaerobe P. A. 3679 (National Canners Association number) are reported in Table 1. The following example will demonstrate the method used in calculating Z values given in the table. For the Z value arrived at

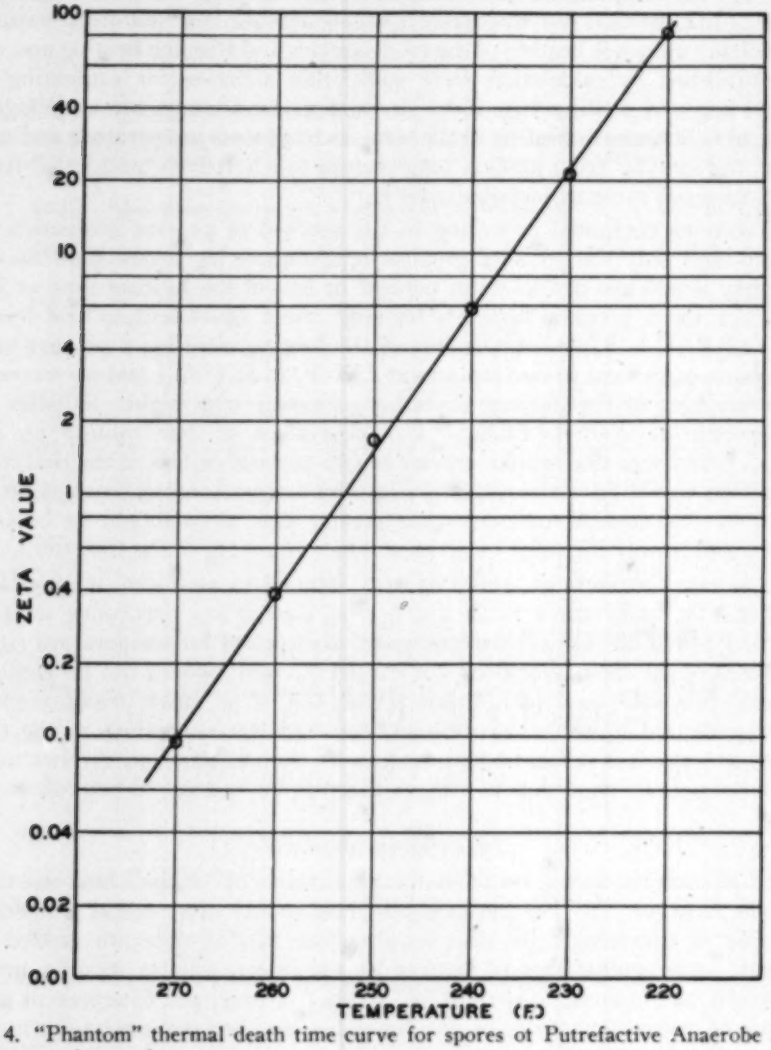

FIG. 4. "Phantom" thermal death time curve for spores ot Putrefactive Anaerobe 3679 suspended in pureed canned peas.

for spores heated at 270° F. (132.22° C.) the values employed to represent terms in the above equation were as follows:

U = 0.393 minutes.

a = 6 (number of samples) x 6000 (number of spores/sample) = 36000 = initial number of spores.

b = 3 = number of spores remaining viable in 6 samples.

Then,

$$Z = \frac{.393}{\log 36000 - \log 3} = 0.096$$

TABLE 1

Thermal Resistance of the Putrefactive Anaerobe 3679 Spores in Pureed Canned Peas

Temperature (° F.)																	
220			230			240			250			260			270		
Heating Time	Number[1] of Positive Tubes	Calculated[2] Z Value	Heating Time	Number[1] of Positive Tubes	Calculated[2] Z Value	Heating Time	Number[1] of Positive Tubes	Calculated[2] Z Value	Heating Time	Number[1] of Positive Tubes	Calculated[2] Z Value	Heating Time	Number[1] of Positive Tubes	Calculated[2] Z Value	Heating Time	Number[1] of Positive Tubes	Calculated[2] Z Value
185.7	6	.....	51.70	6	.....	14.262	6	.....	4.000	6	.....	1.131	6	.....	0.313	6	.....
232.1	6	.....	64.60	6	.....	17.826	6	.....	5.000	6	.....	1.412	5	0.366	0.393	3	0.096
278.5	5	72.21	77.50	6	.....	21.391	4	5.410	6.000	4	1.517	1.697	2	0.399	0.472	0	.....
324.9	5	84.23	90.40	2	21.24	24.957	2	5.865	7.000	4	1.770	1.979	0	.....	0.548	0	.....
371.4	3	91.05	103.40	0	.....	28.522	1	6.260	8.000	1	1.756	2.261	0	.....	0.628	0	.....
Average Z Values																	
82.75			21.24			5.845			1.681			0.383			0.096		

[1] Represents the number of culture tubes in which organism grew subsequent to heat treatment. Each culture tube received an entire sample which before heating contained approximately 6,000 spores. Six replicate samples were subjected to each time-temperature relationship indicated. All cultures were incubated at 85° F.

[2] Calculated using equation, $Z = U/\log a - \log b$. Each positive culture was considered to represent growth from a single spore only if all of the six tubes receiving replicate samples were not positive for growth of the organism.

As shown by Ball *(4)*, values of Z may be plotted on semi-log paper in the direction of ordinates against corresponding temperature in the direction of abscissae to give "phantom" thermal death time curves. Figure 4 shows the average values of Z appearing in Table 1 plotted against corresponding temperatures. Values of the term "z" appearing in mathematical formulae for process calculation may be ascertained from "phantom" thermal death time curves, z being defined as the number of degrees Fahrenheit required for a thermal death time curve to traverse one log cycle. According to the curve in Figure 4, the value of z for spores of P. A. 3679 in the pureed canned peas is 17.0° F. (9.44° C.).

Values of Z for any process temperature in the range covered may be taken directly from thermal death time curves similar to that appearing in Figure 4. Such values are subject to some error because of the small number of samples on which each point used in the establishment of such a curve is based; however, the values should be sufficiently reliable for use in calculation of commercial processes. Naturally the accuracy of Z values may be increased by using a greater number of replicate food samples to study the effect of each time-temperature relationship, for example, 12 or 18 instead of six as employed in studies on which the curve in Figure 4 was based.

Having values for Z, the time required to reduce any number of spores to any other number may be readily calculated. From Figure 3 we find Z = 0.745 for spores of the putrefactive anaerobe 3679 in pureed peas heated at 255° F. If, for example, in calculating a process it were necessary to know the time required at 255° F. (123.89° C.) to reduce 10,000 spores to 0.01 spore, the time (U) required could be determined as follows:

$$U = Z\ (\log a - \log b)$$
$$U = 0.745\ (\log 10{,}000 - \log 0.01)$$
$$U = 0.745\ (4 + 2) = 4.47 \text{ minutes}$$

The significance of rate of destruction data (Z values) in process calculation has been discussed elsewhere *(9)*.

DISCUSSION

The technique herein described yields reliable thermal resistance data for the spores of highly heat resistant bacteria subjected to temperatures as high as 270° F. (132.22° C.). Heating of the medium (containing the spores) to process temperature and cooling it to a temperature having comparatively little lethal value are accomplished so rapidly that errors due to temperature lag are virtually eliminated. The method makes possible for the first time experimental study of thermal resistance of bacteria to temperatures in the range 250° F. (121.1° C.) to 270° F. (132.22° C.). Furthermore, it yields more accurate data for temperatures between 220° F. (104.44° C.) and 250° F. (121.1° C.) than have been obtainable by either the TDT can or TDT tube method because it eliminates the necessity for making corrections for temperature lags during heating and cooling, corrections which on the basis of present knowledge cannot be accurately determined.

The method has the added advantage of comparative simplicity of operation. The fact that the food container does not have to be hermetically sealed makes preparation of the inoculated food samples relatively simple. Because in removing each open container of sample from the thermoresistometer it falls directly into a tube of culture media, the laborious task of subculturing is entirely eliminated. Two additional advantages are inherent in the elimination of subculturing, namely, the

entire sample is always cultured, and the chances for contaminating the processed sample or the culture medium are greatly reduced.

One technician after a week of training can, in one eight hour day, if the spore suspension and culture medium are available, process 216 samples of which a different lot of 36 is subjected to each of six temperatures at five degree intervals from 245° F. (118.33° C.) to 270° F. (132.22° C.) inclusive. This number of samples allows for six replicate samples to be subjected to each of six time-temperature relationships for each of the six temperatures employed. During the eight hours the one technician can prepare the 216 inoculated food samples, process them, layer each culture with sterile agar or other sealing material, and place all cultures in the incubator. Beyond the fact that data collected by methods heretofore available have been subject to considerable error when the methods were applied to studying thermal resistance of bacteria to temperatures in this higher range, it has required about four times as long, to collect a given amount of data as is required by the method described here. Because of this time element, the thermoresistometer will permit of many studies being made which heretofore have been virtually prohibited. The method not only permits a study of the effect of higher temperatures than other methods allow, but it also makes possible a more accurate evaluation of many factors influencing thermal resistance and germination of spores than has heretofore been possible.

Studies designed to determine the nature of thermal death time curves for certain food spoilage types of bacteria in a variety of food products are now in progress. Though these studies are scarcely more than begun, it may be said that for spores of the putrefactive anaerobe 3679 suspended in several different food products the semi-logarithmic thermal death time curves, for temperatures in the range 220° F. (104.44° C.) to 270° F. (132.22° C.) are straight lines. The curve in Figure 3 for the spores in pureed peas is characteristic of those obtained for the spores in the other products studied to date. Values of z thus far determined have been of the order of 17° F. (9.44° C.) to 20° F. (11.11° C.) Studies designed to determine the possibility of applying the technique described to the determination of spore resistance to even higher temperatures, are also in progress. Limited results thus far obtained show great promise for extending use of the technique to the determination of spore resistance to temperatures as high as 290° F. (143.33° C.). It is the intention to publish, in the near future, further results of these studies along with the bacteriological methods employed.

SUMMARY

A technique developed for studying thermal resistance of bacterial spores to temperatures in the higher range, 240° F. (115.56° C.) to 270° F. (132.22° C.), has been described. Although the technique was specially developed for studying effects of these higher temperatures, it is applicable to the study of spore resistance to any temperature between 215° F. (101.67° C.) and 270° F. (132.22° C.).

By the technique described, spore resistance to temperatures in the higher range may be determined with an accuracy far exceeding that obtainable by any method heretofore described. In addition, determinations may be made in approximately one-fourth the time required by other available methods, and with far less effort.

Methods, for treating spore resistance data obtained by the new technique, by which essential values for calculating thermal processes for foods may be ascertained have been described in full. A small amount of thermal resistance data has been included to demonstrate the nature of data obtainable and the method of treating data.

ACKNOWLEDGMENT

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A RATIONAL METHOD FOR GRADING FOOD QUALITY[a]

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Numerical grades for any single food property should be proportional to the quality expressed in descriptive terms. The grades of single properties should be "weighed" by using specific multiples and then added to form a total grade. Total subjective and objective grades should be combined with biological value and external state of a commodity to calculate the final grade, again using specific multiples. This final grade would be a measure for integral quality.

1. INTRODUCTION

It seems to be widely recognized by the U. S. Department of Agriculture and by food experts that quality labeling of food is necessary for the protection of the consumer and for price regulations. It is still a question, whether a numerical grade judgment or a descriptive judgment would be better adapted for this purpose. There is no doubt that a descriptive method could cover a wider range of properties and reveal more detailed information. But to be satisfactory it needs a very extensive and complete evaluation. Grading always means the replacement and expression of quality by quantity. For such a complicated conception as *quality of food*, which depends upon a large number of characteristic properties, grading may always contain some amount of inaccuracy and arbitrariness, but there is no doubt that it has the advantage of conciseness and simplicity.

Although food is a very complex substance, the application of numerical grades to its examination can be justified, as such grades are generally adopted, even in schools, for judging the mental capacity of human beings, who of course are very much more complicated subjects than food.

Any use of a number, to express the result of a measurement or of an estimation, should be made in conformity with some elementary arithmetical laws. No rational grading system should contain points contradicting with fundamental arithmetical conceptions. In any grading, for instance in the definition of the thermometric scale, or in the measurement of any other property, there is always a certain amount of arbitrariness with regard to:

1. the selection of some fixed points on the scale corresponding to some standard values of the property in consideration,

2. the selection of an appropriate unit. As an example we may remember that in temperature measurements on the centigrade scale we consider arbitrarily the freezing point and the boiling point of water at ordinary pressure as fixed points. Attaching to them the values zero and 100 respectively, we divide this interval in 100 *equal* parts and call each part "one degree." If temperature is measured by the thermal expansion of a substance, e.g. mercury, each degree corresponds to the same increase in volume or in length of a column.

2. CHARACTERISTICS OF A RATIONAL GRADING SYSTEM

The fundamental supposition of any rational quality grading is that the number expressing the grade be proportional to the quality of the property to be measured. In other words, an equal difference in grade numbers must correspond with an equal difference in quality. This makes it self understood that high qualities should

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correspond to high grade numbers. Any deviation from this rule is always connected with a false correlation between grade and quality.

To make this point clear let us assume that we select the number 100 (points or percent) for the highest possible quality of a commodity, corresponding to the denotation "perfect." We should use the full scale between 0 and 100 to express relative qualities. It is now arithmetically sound (and therefore rational) to attach the mean number 50 to a mean (medium or average) quality. Anyone will then understand that the numbers between 50 and 100 correspond to quality degrees between mean and perfect, and numbers between 50 and 0, to quality degrees between average and extremely poor or not eatable. The exact correlation between grade number and quality denotation may still contain some amount of arbitrariness, but it should not be too difficult to find a general agreement upon such grades. The difficulty of such a correlation lies in the proper selection of the words expressing the quality, these words not always having a definite and precise meaning. But the permissible scope in the interpretation of a term is never so big as to make impossible the correlation between a term and a grade number.

TABLE 1

Quality Term		Quality range in percent	Grade
First Alternative	Second Alternative		
Perfect (fancy)	Excellent	over 95%	10
Excellent	Very good	85 to 95%	9
Very good	Good	75 to 85%	8
Good	Slightly good	65 to 75%	7
Slightly good	Borderline plus	55 to 65%	6
Average (mean)	Borderline minus	45 to 55%	5
Fair	Slightly poor	35 to 45%	4
Borderline	Poor	25 to 35%	3
Bad (defective)	Very poor	15 to 25%	2
Very bad	Extremely poor	5 to 15%	1
Unacceptable (not eatable)		below 5%	0

Let us demonstrate such practical correlations offering two examples. The first alternative (first column in Table 1) was proposed by J. Rheinhold *(8)* for determining the quality of fruits and vegetables (with the only difference that his grades have been proposed in the inverse order, 0 for the highest quality and 10 for "not eatable"). The second alternative (second column of table 1) was used as a scoring system for meat by J. M. Ramsbottom *(7)*. Both schemes are quite reasonable, as each step expressed by a decrease of one unit in grade corresponds with a quality decrease of practically equal descriptive value. We may agree that the step from very good to good (from grade 9 to 8) is equal to the step from poor to very poor (from grade 3 to 2). As two successive grades always differ by one unit, the terms used to describe two successive qualities should be selected so as to express equal differences in sensation.

Such a terminology may contain a certain amount of arbitrariness, which can only be avoided by accepting "definitions." The grades can then be easily correlated with some percentage in quality, provided that the whole scale of grades, from zero up to the selected highest value (10 in table 1) is utilized. Such quality percents are indicated in the third column of table 1. As the grades are discrete numbers and quality may be regarded as a continuous property, it becomes clear that each grade corresponds with a quality region. So grade 5 and the descriptive term "mean or average" do not just mean 50% quality, but are valid within a range of $50 \pm 5\%$,

thus covering a region of 10% of the continuous quality scale. But the two extreme grades 10 or 0 embrace only the highest or lowest 5% of quality. Such fluctuations appear in any subjective judgment, which is always connected with an amount of uncertainty. They are also admitted in any biological consideration.

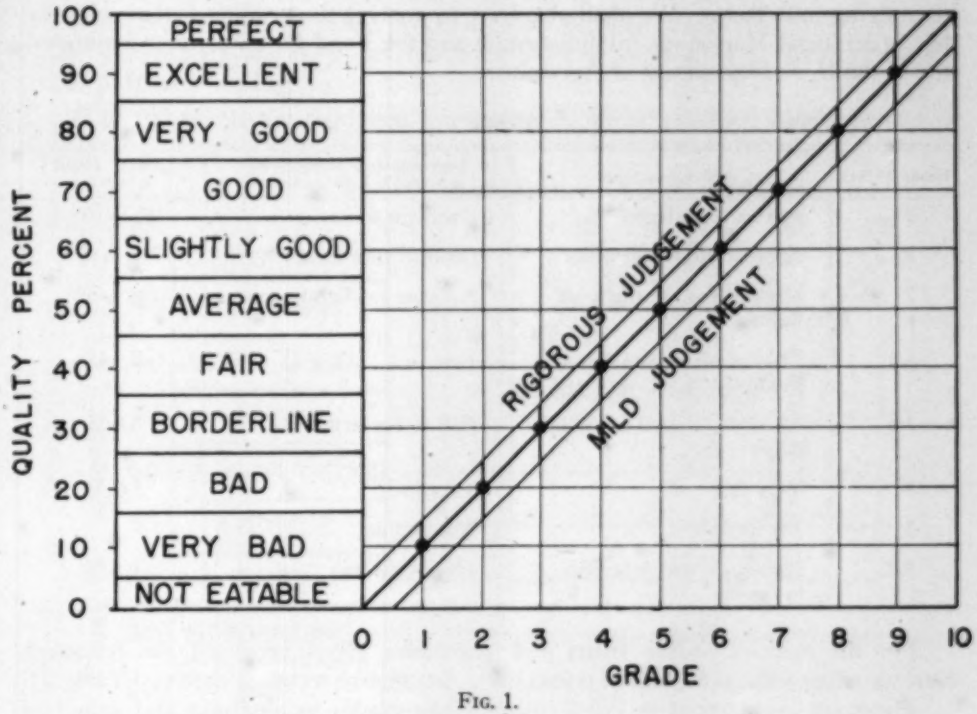

FIG. 1.

The values of Table 1 are graphically represented in Fig. 1. The heavy straight line correlates the grades 0 to 10 with the mean values of each quality region. Two other parallel lines are inserted in the figure, in a vertical distance of + 5 and of — 5% from the main line. The upper line represents the results of a rigorous judgment, the lower line—those of a mild judgment. A mild judge may concede the grade 6 to a 55% quality, whilst a rigorous judge would request for this grade a quality of 65%, grading 55% with 5. The area between the upper and the lower line is the region of fluctuations or uncertainty. Even the best trained judge might not be able to reproduce his judgment within a narrower range than ± 5% of quality. Therefore it seems to me that using 10 grades, it is sufficient to grade partial food properties in integer numbers and not to introduce decimal fractions. Combined or derived values of grading, which will be discussed later, may contain such fractions.

The fluctuation of ± 5% may be regarded as a tolerance, and it is certainly questionable whether this amount is well chosen and can be realized in testing food. My personal experience shows that ± 5% is quite right in laboratory work with several precautionary measures, and with well trained tasters. Generally a larger range of uncertainty up to ± 10% should be granted and then a scale of 5 grades from 0 to 5 could cover the whole field, each grade corresponding to a region of 20% quality. The extension of the region of fluctuations may be fixed by a board

of food experts. A scale of 10 grades would very much simplify the subsequent calculations.

To show how much some adopted systems of grading differ from the rational requirements developed above we may mention a scale proposed by Heiss *(3)* for frozen fruits and vegetables, and another one suggested by Babcock and Leete *(2)* for judging milk flavor. We shall also have to analyze the grading system used by the Agricultural Marketing Administration and the Food Distribution Administration of the U. S. Department of Agriculture.

TABLE 2

Grade Points	Descriptive term	Corresponding terminology from table 1, first column	Quality range in percent
9	Just as fresh fruits	Excellent to perfect	85 to 100
8	Approaching fresh fruits	Good to very good	65 to 85
7	Slightly flat, or slight off flavors	Average to slightly good	45 to 65
6.5	Flat, or off flavors, but still eatable	Fair	35-45
5-6	No more saleable due to off flavor	Bad to borderline	15-35
4	Very bad	Very bad	5 to 15
3	Not eatable	Not eatable	
0-2	Extremely bad, injurious to health	(Not foreseen)	0 to 5

For the taste of frozen fruits and vegetables Heiss proposed the following corresponding values of grades (points) and descriptive terms of quality (Table 2).

These are represented in Fig 2, using Heiss-grades as abscissae and values of our corresponding descriptive terminology for quality percentage (see Table 1) as ordinates. We see that the line correlating grade and quality deviates very much from the desired straight line. The main reason for this disagreement is, that too many grades are attached to the lowest qualities (0 to 3). One unit difference in grade is not at all related to an equal step in quality in different parts of the scale.

The same objections must be raised against the grading of milk flavor proposed by Babcock and Leete: in their scale the perfect score is 25, milk that scores 23 or above is designated as excellent, 21 or 22 as good, 18, 19, 20 as fair, 12 to 17 inclusive as poor and 11 or below as bad. The corresponding curve is also shown in Fig. 2, it does not differ very much from that of Heiss. It is difficult to conceive why 11 grades from 25 are necessary to express bad qualities. Grade 10 and grade 5, both in the "bad" region show certainly in this scale a smaller difference in quality than say grade 21 (good) and grade 16 (poor) although in both cases there is a grade-difference of 5 units.

The grading system used by the Department of Agriculture for a great number of commodities may be illustrated in its application to peaches *(10)*. Five single properties are judged:

1. Uniformity of color, best grade 20 points
2. Absence of defects, best grade 15 points
3. Maturity, best grade 40 points
4. Tenderness, best grade 10 points
5. Flavor, best grade 15 points

Total, best grade 100 points.

Thus 100 points correspond with perfect quality and one should expect that 50 points be attributed to an average quality, which represents the bulk of peaches produced at present. Any quality higher than this average should reach more than 50 points, but qualities below average, reaching only 40 or 30 points may still be sold on the retail market at lower prices, or they may be used for industrial products.

In the system adopted now a proportionality between grades and quality is maintained between 100 and 60 points, following more or less a straight line approaching "mild judgment" in Fig. 1:

90 to 100 points are requested for quality A, which certainly means perfect or fancy.

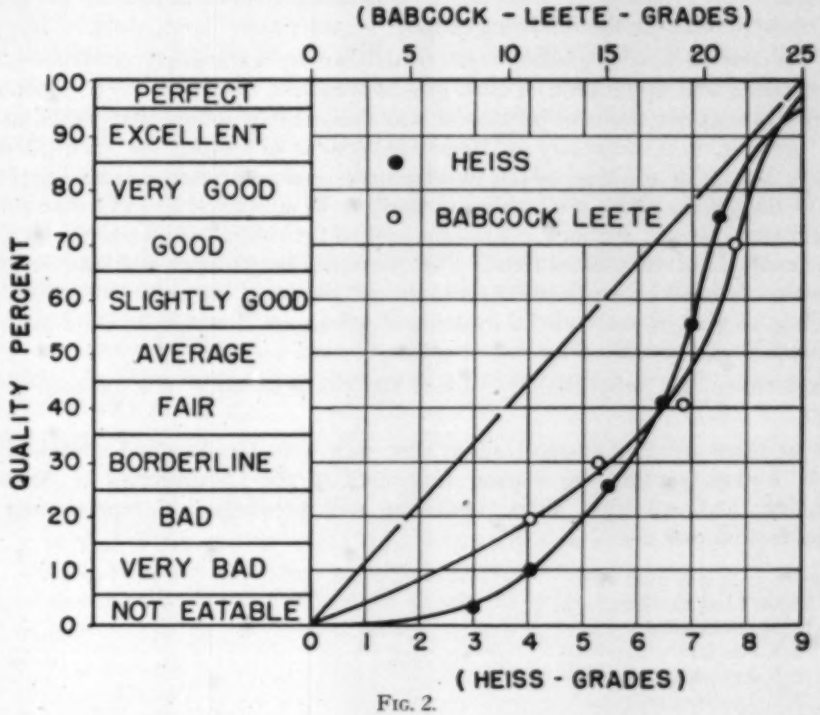

FIG. 2.

75 to 90 points are requested for quality B, which means "choice," excellent or very good.

60 to 75 points are requested for quality C, which still means good, or standard.

Less than 60 points represent substandard qualities. Points below 60 are no more clearly distinguished, and corresponding qualities are not exactly formulated. Food below 60 points may still be sold but must be labeled as follows:

"Below standard in quality, good food—not high grade."

Here again the attribute "good" appears, which is confusing. What is the difference in quality and price between 59 points and say 40 points. How many points stay on the line of demarcation between saleable and no more saleable food? In the present system more than half of the scale (0-60) is not defined.

There are certainly no objections to introduce the conception of a "standard quality" and to fix arbitrarily its lower limit. This limit may even be sliding and subject to changes, depending on one side on improvements in production and processing of food, and on the other side on the degree of wealth and prosperity of

a country. In periods of abundance and development the lower limit of "standard quality" may deliberately be fixed at a grade which is more or less above "average quality," that is above 50 percent, or above grade 5 on a 10-grades scale. This is at present the case in the grading system of the Department of Agriculture, where this limit was fixed at 60 percent (points) that is in the region of good. I am sure that postwar conditions in European and Asiatic countries justify a lower limit of "standard quality" by no means higher than 50 percent and rather 40 percent. But even lower qualities should be related to definite grades; they may be used for minor food products, for animal food, etc.

M. B. Jacobs in his comprehensive book "The Chemistry and Technology of Food and Food Products" *(4)* discusses the grading system adopted by the Department of Agriculture in the following terms:

"Different systems of grading were established for various commodities and the interpretation and application of each grades were not uniform. . . . Since grade nomenclature is not uniform because it was designed to follow the grades used in trade practice, it is necessary to learn the system of grades for each particular product. . . . In addition to the development of new grades we can expect that some of the grades which are in use are likely to be simplified or otherwise changed as time goes. Recent changes in grading beef to provide a simpler terminology are a good example of the present trend. The consumer wants more and simpler grades. When this demand becomes sufficiently strong, the trade interests will undoubtedly be willing to give up their varied systems of grading and adopt A, B, C or 1, 2, 3, systems in favor of uniformity and simplicity."

Uniformity and simplicity are indeed the characteristics of a rational grading system.

Some other grading systems agree very well with the diagonal straight line in Fig. 1. I may mention the scoring scale used in the Laboratories of Arthur D. Little, Inc., at Cambridge, Mass., in testing milk powders, and representing commercial tasting practice:

A 15-point scale was used with
15, 14, 13 as excellent
12, 11, 10 as good
9, 8, 7 as fair
6, 5, 4 as poor
3, 2, 1 as very poor.

It is reasonable to assume that the difference in quality between good and fair is equal to that between fair and poor, and that also

excellent minus good = poor minus very poor

thus this scale is indeed practically linear and fills our demand.

A large number of other scales proposed in different countries for different commodities has been explained and criticized in a previous paper *(8)*.

We strongly advocate the general acceptance and practical use of a grading scale, in which the number representing the grade is proportional to the quality.

Quality is expressed in descriptive terms which can always be selected so as not to contradict appreciably the desired proportionality as shown in Table 1. Whether the scale should be restricted to 10 or 5 grades, or extended to 15, 25 or even 100 depends on the sense-thresholds of the tasters and may be decided by experts. The author's experience shows that 10 integer numbers are sufficient for the quality determination of any single property (such as taste, flavor, color, tenderness). The

total grade expressed in terms of such single property grades, may then contain decimal fractions.

3. SELECTION OF PROPERTIES (FACTORS)

The selection of essential single properties is certainly quite different for different commodities. We already mentioned 5 properties, which have been adopted in determining the quality of peaches (and of other fruits). For meat Ramsbottom *(7)* graded the following properties: appearance (lean and fat), aroma, flavor (lean and fat), juciness and tenderness. Dairy products may be characterized by other properties, wines again by other. As soon as an agreement is reached as to the grade-numbers, the same numbers should be used for all selected properties. Using the grades 0 (or 1) to 10, explained in Table 1, grade 6 should express a "slightly good" flavor, a "slightly good" tenderness, a "slightly good" uniformity of color and so on. It is absolutely unsuitable to use different grade numbers for the same descriptive term in different properties. But such differences in numbers for the same descriptive term become unavoidable if different "best points" are introduced, for different properties, as was shown in the example of peaches.

It is obvious that not to all of the properties selected for a special commodity can be awarded the same "weight" in determining the total quality and in calculating the total grade. So we have to introduce *"specific multiples"* for each property and multiply the grade number with the corresponding "specific multiple."

Properties and "specific multiples" must be determined for each commodity by a board of prominent experts. The arithmetical evaluation of the total subjective grade G_s is much facilitated, if the sum of all values of the different specific multiples m equals 10. The very simple formula for the evaluation of the total grade G_s, using the grades g_1, g_2, g_3 . . . and the specific multiples m_1, m_2, m_3 . . . of the single properties is

$$G_s = \frac{\Sigma\,(g \times m)}{\Sigma m} \qquad . \quad . \quad . \quad . \quad (1)$$

Σ is the symbol of summation which is to be extended over all selected single properties. The use of formula (1) may be explained on an example:

In a previous work on grading the quality of frozen food (6) the author has selected the following properties and attached to them the following specific multiples:

1) Taste (flavor)	$m_1 = 4$
2) Aroma (odor)	$m_2 = 2$
3) Consistency*	$m_3 = 2$
4) Color	$m_4 = 1$
5) Form (appearance)	$m_5 = 1$
	$\Sigma m = 10$

Now if on a 10-grade scale (such as in Table 1) the result of testing was expressed by the grades $g_1 = 8$, $g_2 = 10$, $g_3 = 6$, $g_4 = 8$, $g_5 = 6$ then the total subjective grade, according to equation (1) is

$$G_s = \frac{8 \times 4 + 10 \times 2 + 6 \times 2 + 8 \times 1 + 6 \times 1}{10} = 7.8$$

The total grade G_s is evaluated on the same 10-grade scale as the single properties; a value 6 would again express a "slightly good" quality. But the value of G_s may contain a decimal fraction.

*Other terms may be used for this property, such as viscosity, turgidity, tenderness, texture.

In Table 1 the grade 0 (zero) was omitted in the second alternative. This grade is indeed not very important. But its introduction can be coupled with a special reservation: if any single property is graded zero, then the total quality should be termed "unacceptable" and the formula (1) should not be applied. That means that grade 1 for some property can be compensated by high grades of other properties, but grade 0 can no more be compensated, and should therefore be used by a judge only to express the unconditioned rejection of a sample.

The "point system" very much used in this country is in some respect equivalent to the system proposed in this paper, but it is not as uniform; in the point system a certain quality, say "good" is expressed by different numbers for each property and for the total, the highest number of points always expressing perfect quality. This can be seen from the example given in paragraph 2 for peaches. It should be realized that for a change from the point-system to our grade-system one should use the equation

Number of points = grade $\times$ specific multiple = $g \times m$.

Thus a point system with 100 points expressing perfect quality is equivalent with a grade system of 10 grades combined with specific multiples m satisfying the condition $\Sigma m = 10$. In the point system mentioned for peaches the corresponding specific multiples for the passage to our grade-system are

1. For uniformity of color	m_1	= 2
2. For absence of defects	m_2	= 1.5
3. For maturity	m_3	= 4
4. For tenderness	m_4	= 1
5. For flavor	m_5	= 1.5
	Σm	= 10

In a grade system the judges have only to keep in mind a scale similar to that shown in Table 1. They know once for ever that "good" is expressed by grade 8 and "fair" by grade 4. They have to apply these numbers for any property. The calculation of total grades, using equation (1), can be done later by a mathematically trained person, introducing for each property the specific multiples upon which an agreement has been reached. These multiples can even be changed as time goes on (always satisfying the condition $\Sigma m = 10$) if new experience suggests such a change, and the resulting total quality can be recalculated using the original grade-numbers of the judges. In the resulting grade the number 8 again expresses "good" quality and the number 4 "fair" quality. Such a uniformity simplifies very much the understanding of a grading system even by non-trained persons.

In the opinion of the author the 5 properties used above for the definition of the total quality of peaches have not been selected very well. The properties should be more or less independent of each other and they should be defined precisely. What means "absence of defects"? Of which defects? Tenderness and flavor depend completely on maturity. It would be reasonable to exclude maturity as a single property, as it has already the characteristic of a complex property. Tenderness and flavor should then be weighed higher and besides this the property "aroma" should be added.

A much better property selection was made by Woodroof and Shelor *(12)* in determining the quality of frozen packed chicken. This is:

Appearance	15 points	corresponding	m_1	= 1.5
Aroma	10 points	corresponding	m_2	= 1
Color	15 points	corresponding	m_3	= 1.5
Texture	20 points	corresponding	m_4	= 2
Flavor	40 points	corresponding	m_5	= 4
			Σm	= 10

The selection of the single properties and the determination of the values of specific multiples is the most responsible task to be performed for each commodity.

4. SUBJECTIVE AND OBJECTIVE TESTS

In a previous paper *(6)* the author has stressed that there is a tendency to replace more and more the subjective (organoleptic) judgments, based on physiological sensation and psychological evaluation, by objective physical or chemical measurements. There is no doubt that any subjective judgment is not only connected with a certain amount of uncertainty, depending on the threshold values of our senses, but that it contains also the reflection of individual elements of different judges. The same objective amount of tenderness or texture (of a commodity) may not please different persons equally well. Even the same person may react in a different manner on the same excitation, depending on different physical and mental disposition.

Many efforts have been applied to find objective methods for measuring the different properties of foods. It is not the purpose of this paper to mention and to criticize all of them; this was done some time ago in a very comprehensive paper by Howard R. Smith *(9)*. The problem is not unsoluble for such qualities as size, color or texture. But it becomes substantially complicated for taste (flavor) and odor (aroma). All present attempts for an objective determination of these properties may be regarded as the first staggering steps on a long and tortuous way. Subjective judgments of food quality still represent the decisive characteristics, and additional objective measurements may previously be given the significance of a controlling and supplementing method. The author agrees with the opinion expressed by Lowe and Stewart *(5)* that in actual practice subjective and objective tests should be used simultaneously. Jacobs *(4)* expresses doubt, "whether there will ever be any method developed which eliminates the personal element in grading food. The connoisseur will always be the final authority in the judgment of the finer qualities of flavor and aroma." As long as we do not know anything about the physical nature of taste and odor we cannot hope to perform correct physiological or psychological measurement. As in the matter of light and sound physical understanding will be the first step of physiological measurements.

In the present state of development objective methods of determining and grading food quality may be regarded as very useful tools supplementing and controlling but not replacing the subjective (organoleptic) tests. In estimating the comparative value of these two methods the subjective test based on immediate reactions of our senses must still be given a higher weight. Expressing this view in terms of this paper we should say that to the total subjective grade G_s calculated in equation (1) should be attached a higher "specific multiple," than to the total objective grade G_o, which can be calculated in the same way, using the grades found for single properties by means of objective measurements.

5. BIOLOGICAL VALUE

It seems reasonable to include in this conception all properties determining the nutritive value of foods together with a microbiological examination consisting in counts of bacteria or yeasts, and in microscopic observations of moulds. Determination of enzyme content (e.g. catalase in frozen fruit) is equally important as enzymes cause decomposition (autolysis) and thus determine the keeping quality of food in storage *(11)*.

The nutritive value may be expressed by the amount of the following substances:

1. Proteins
2. Carbohydrates and organic acids
3. Fats
4. Minerals
5. Specific substances (vitamins)

The microbiological examination reveals the degree of freshness or of deterioration. For example bacterial counts on fish permit to estimate its condition and to predict its keeping quality. The limit of usefulness is very often determined by the presence of a certain number of microorganisms.

The evaluation of the biological value can be completed by a hygienic or sanitary inspection with regard to the presence of pathogenic micro-organisms.

The different properties mentioned in this group may again not have the same significance. Therefore, specific multiples should be used in the same way as was shown in the previous paragraphs and a total biological grade G_b should be calculated with the use of equation (1).

6. EXTERNAL STATE

A last group of properties can be assembled in what may be called the external appearance of a commodity. It includes the first subjective optical impression when a food is served and a series of objective judgment as uniformity of size, freedom from undesired substances (pips, stones, stalks, skin) cleanliness and also the quality of packing or wrapping. The grades of all these partial qualities combined with their specific multiples should again be condensed to a total external grade G_e using equation (1).

7. GRADING THE INTEGRAL QUALITY

Special attention should be called to the fact that grades of biological properties are based on exact measurements, just as was the case with the objective determination of color or texture. This makes it much easier to connect quality with numerical grades, as correlations are replaced here by distinct functions (proportionalities). If, for example, some fresh fruit has a certain content of vitamin C, which we may consider as 100%, and we find in a frozen package prepared from this fruit 64% of the original amount, then the grade of this property on a 10-grade scale is certainly 6.4. Another method of grading the vitamin C content would consist in fixing normal values, as proposed by Tressler for carefully handled frozen vegetables *(11)*. In these cases it must be allowed to use decimal fraction even for single properties. A fluctuation of $\pm 5\%$, admitted in Fig. 1 for subjective judgments is no more justified in objective measurement, which generally can be performed with a higher precision.

In estimating the quality of food we have now arrived to four total grades G considering for categories:

1. The subjective (organoleptic) quality ..... graded G_s
2. The corresponding objective quality ..... graded G_o
3. The biological value ..... graded G_b
4. The external value ..... graded G_e

Using the same method as with single properties, that is introducing "specific multiples" we can now determine a final or integral grade G for the commodity. There must of course be found an agreement as to the values of the specific multiples for the four total grades. It is again practical to select such multiples whose sum will be 10.

We feel that in the present state of our quality control the subjective grade G_s should be given the highest weight and we propose for it a specific multiple

$m_s = 4$ or 5. The corresponding objective grade may be assigned $m_o = 1$ or 2, depending on the development and general acceptance of the measuring methods employed. For the biological grade we propose $m_b = 3$ and for the external grade $m_e = 1$. The final or integral grade is then

$$G = \frac{\Sigma(G \times m)}{\Sigma m}$$

For example with $G_s = 7.8$; $G_o = 7.0$; $G_b = 6.2$ and $G_e = 8.5$ we find

$$G = \frac{7.8 \times 5 + 7.0 \times 1 + 6.2 \times 3 + 8.5 \times 1}{10} = 7.31$$

The last decimal fraction may be omitted.

The exact determination of such a final grade for the quality of foods may not yet be possible as not all properties entering this complex conception can at present be graded with sufficient accuracy. For the time being we may aspire to a most correct and reproducible determination of G_s and content ourselves with approximate values of the other total grades.

Further research should mainly be devoted to a more exact determination of G_o and G_b.

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SOME RESULTS OF HORMONE TREATMENT OF FIELD-GROWN TOMATOES [a]

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A comparison was made of the composition of tomatoes grown on hormone-treated, emasculated-hormone-treated, and control tomato plants grown in the open field. The nutritive value of the hormone-treated (β-naphthoxyacetic acid) tomatoes did not prove sufficiently superior to that of the controls to justify their production.

During recent years studies have been made of the effect of treating growing plants with numerous hormones. Murneck, Wittmer, and Hemphill *(7)*, concluded from several years' experiments that hormone will promote the setting and increase the size of greenhouse tomatoes. They found p-chlorophenoxyacetic, β-naphthoxyacetic, and 2, 4-dichlorophenoxyacetic acids most effective. Zimmerman and Hitchcock *(12)* have stated that 2, 5-dichlorobenzoic acid, 2, 4-dichlorophenoxyacetic acid, 2-chlorophenoxypropionic acid, and β-naphthoxyacetic acid were especially valuable for inducing parthenocarpy. Johnson *(6)* produced greater vegetative and reproductive response in tomato plants with o-phenylenediacetic acid than with phenoxyacetic acid or β-naphthoxypropionic acid. Roberts and Struckmeyer *(8)* secured a good set of tomatoes by spraying tomato flowers with a 75 mg. per liter solution of 2, 4-dichlorophenoxypropionic acid but the hormone produced foliage distortion. In attempting to increase the fruit set of tomato plants, Bretz *(2)* volatilized dichlorophenoxyacetate in a greenhouse and caused marked distortion and twisting of the stems and leaf petioles, blistering of the epidermis, profuse initiation of adventitious root primordia underneath the epidermis of the stems and marked distortion of the fruit. When Swarbrick *(11)* applied a 0.001% solution of naphthaleneacetic acid to the flowers, fruit development of both tomatoes and strawberries was delayed, and a marked twisting of the petioles resulted. Swarbrick also reported that while one spray at full flower, with 0.001% naphthaleneacetic acid delayed the ripening of strawberries twenty-one days, a spray with 0.001% naphthoxyacetic acid greatly improved the fruit set and increased the commercial crop by one-third. Obviously, the proper concentration of hormone must be determined for each chemical, for each variety of plant, and for the purpose desired since improper application or concentration may cause disastrous results.

It is generally believed that the tomato seeds consumed in tomatoes are affected little if at all by human digestive juices. Thus, although tomato seeds contain valuable constituents, they contribute little or nothing to human nutrition. Accordingly, one may question whether or not tomatoes, made seedless by hormones, possess the same nutritive value as corresponding normal seed-containing tomatoes. In an earlier study Holmes, Spelman, Kuzmeski, and Lachman *(5)* examined tomatoes grown in a typical greenhouse during the winter months and found essentially the same amounts of water, carotene, and magnesium in the hormone-treated tomatoes, and in the controls. The treated tomatoes contained somewhat more nitrogen, phosphorus, potassium, and calcium, but less reduced ascorbic acid than the controls. While it is possible to provide the same soil and fertilizer for both the greenhouse and the open field, there is a pronounced difference in the amount and intensity of

[a] Contribution No. 654 Massachusetts Agricultural Experiment Station.

sunshine received by tomato plants grown in a greenhouse in this latitude in the winter months, and those grown in an unshaded field during June, July, and August. This study was conducted to accumulate data regarding the composition of hormone-treated tomatoes grown in an open field during the summer months.

EXPERIMENTAL

A standardized Pritchard, Harris strain tomato was used in this study. The seed was sowed on April 10 in flats in the greenhouse. Six weeks later, May 28, well-developed plants were set out in an unshaded field. The plants were set eighteen inches apart in rows that were four feet apart. During the entire season the area between the plants was kept free from weeds and other plant growth. Thus all the available plant food was conserved for the tomatoes. The plants were staked and pruned—all side branches being removed as soon as they appeared. The fertilizer, which was a 5-8-7 commercial fertilizer,[b] was applied, broadcast, when the plants were set out at the rate of 2000 pounds per acre.

Provision was made for two types of treatments; i.e., (a) hormone-treated plants, (b) emasculated and hormone-treated plants, and corresponding untreated plants to serve as controls. There were three replicates of each. Thus there were twelve groups of tomato plants that were grown under identical conditions except for the emasculation and hormone treatments. The hormone was β-naphthoxyacetic acid and it was used at a dilution of 50 parts per million. It was applied with a nasal atomizer. The first application was made when the first blossoms opened, and subsequent applications were made three times per week as new blossoms opened. The solution of β-naphthoxyacetic acid was sprayed directly into the individual flowers until small droplets of the solution could be seen inside the flower. The procedure for the four groups of plants that were both emasculated and hormone-treated was the removal of the anther cone from each blossom by hand just previous to anthesis.

Since the tomatoes were harvested on August 25, the growing period was 89 days. The meteorological observations for this immediate locality for the period of the experiment have been reported by Sievers *(9)* and Stapleton *(10)*. Between May 28 and August 25, there were 823 hours of bright sunshine and 6.82 inches of rain fall. The mean relative humidity was 68.5% for June, 72.3% for July, and 80.4% for August. The least daily temperature change was 7° F. on June 8 and the greatest was 38° F. on June 23. The lowest temperature was 37°, and the highest 95° F. The mean temperature was 64.6° for June, 73.2° for July, and 73.8° F. for August.

When tomatoes were harvested for assay, nearly one-half bushel was picked from each of the twelve groups of plants. Three samples of twenty tomatoes were assembled for the assays by carefully selecting five typical tomatoes from the control, the hormone-treated, and the emasculated and hormone-treated groups and their respective replicates. The tomatoes were disintegrated and uniformly mixed with a Waring Blendor. Determinations were made for water, total solids, soluble solids, reduced ascorbic acid, carotene, calcium, magnesium, manganese, nitrogen, phosphorus, and potassium. Except for carotene and soluble solids, all determinations were made by the official methods of the Association of Official Agricultural Chemists *(1)*. Campbell's *(3)* tables were used for converting the values obtained with a Zeiss refractometer for refractive index into soluble solids, and the carotene content of the tomatoes was determined by the procedure adopted by Holmes and Spelman *(4)* for squashes.

[b] 5% nitrogen, 8% phosphorus, and 7% potassium.

RESULTS AND DISCUSSION

The data essential for comparing the composition of tomatoes from the three groups, i.e., hormone-treated, emasculated and hormone-treated and controls are reported in Table 1. All three groups contained essentially the same amounts of water, total solids, ascorbic acid, calcium, and magnesium. The emasculated and hormone-treated tomatoes were definitely lower in manganese than the other two groups. The largest amount of potassium was found in the controls, slightly less was found in the emasculated and hormone-treated tomatoes, and considerably less was found in the hormone-treated tomatoes. Also the hormone-treated tomatoes had decidedly less nitrogen than either the controls or the emasculated and hormone-treated tomatoes. The emasculated and hormone-treated tomatoes were richer in carotene and phosphorus than tomatoes from either of the other two groups. The controls contained the least amount of both carotene and phosphorus.

TABLE 1

Comparison of the composition of hormone-treated tomatoes

Sample	Water	Total Solids	Soluble Solids	Ascorbic Acid	Carotene	Calcium
	%	%	%	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*
Control	92.9	7.1	6.83	15	.47	17
Hormone-treated	93.0	7.0	6.83	16	.54	16
Emasculated and hormone-treated	92.2	7.8	7.59	17	.63	16

Sample	Magnesium	Manganese	Nitrogen	Phosphorus	Potassium
	mg./100 g.	*p.p.m.*	*mg./100 g.*	*mg./100 g.*	*mg./100 g.*
Control	15	2.2	179	29	292
Hormone-treated	14	2.2	147	30	276
Emasculated and hormone-treated	15	1.0	185	35	289

Data were accumulated regarding the effectiveness of the hormone β-naphthoxyacetic acid for producing seedless tomatoes. When the tomatoes were harvested each group was examined for the presence or absence of seeds. Seventy-five percent of the hormone-treated tomatoes were seedless and 25% contained seeds. All of the emasculated and hormone-treated tomatoes were seedless. All of the control tomatoes contained seeds, but in 52% of the tomatoes there was some abnormality in the grouping of the seeds in the placenta. Although the β-naphthoxyacetic acid solution used in this study of open field, summer grown tomatoes was only two-thirds as concentrated as that used by Holmes, Spelman, Kuzmeski, and Lachman *(5)*, the number of seedless tomatoes produced was 50% greater. However, in making this comparison it should be remembered that a number of factors were not identical; i.e., climatic conditions, amount of sunshine, and variety of tomato. In the greenhouse study, Waltham Forcing variety was used, and in the open field study, Pritchard was planted. Zimmerman and Hitchcock *(12)* found β-naphthoxyacetic acid was particularly effective in inducing parthenocarpy. In a study of several hormones, Murneck, Wittmer and Hemphill *(7)* concluded that β-naphthoxyacetic acid was one of the most effective of those tested. While emasculating tomato plants by hand proved most effective for producing seedless tomatoes, that procedure is hardly

practicable for tomatoes grown on an acreage basis. The results obtained in this study with field-grown tomatoes, and those obtained by Holmes, Spelman, Kuzmeski, and Lachman *(5)* with greenhouse tomatoes do not reveal a significant difference in the over-all composition of control and hormone-treated tomatoes. Thus, considered on the basis of their nutritive value, the hormone-treated tomatoes are not enough superior to the controls to justify the extra labor and expense involved in their production.

SUMMARY

The compositions of tomatoes grown on hormone-treated, emasculated and hormone-treated, and control tomato plants have been compared. The plants were grown under open field, summer conditions. Well-developed standard Pritchard plants were used. The soil, fertilizer, rainfall, sunshine, climatic and cultural conditions were identical for the three groups, and the three replicates of each. Three hormone treatments with a 50 parts per million solution of β-naphthoxyacetic acid were made weekly. As fast as tomato blossoms opened, the hormone was applied with a nasal atomizer until droplets could be easily seen within the flowers. The emasculated and hormone-treated plants were prepared by removing the anther cone by hand just previous to anthesis, and immediately spraying with the hormone. The tomatoes were harvested 89 days after the plants were set out. Assays were made for water, total and soluble solids, ascorbic acid, carotene, calcium, magnesium, manganese, nitrogen, phosphorus, and potassium. The three groups of tomatoes contained similar amounts of water, total solids, ascorbic acid, calcium, and magnesium. The controls contained the most potassium. The emasculated and hormone-treated tomatoes contained the least manganese, but the most carotene, nitrogen, and phosphorus. All the emasculated and hormone-treated tomatoes were seedless, whereas 25% of the hormone-treated, and all the controls contained seeds. Judged by these data, the nutritive value of the hormone-treated tomatoes is not enough superior to that of the controls to justify their production.

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FOOD FOR INFANTS, INVALIDS AND THE AGED*

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An attempt was made to obtain palatability in strained foods by judicious combinations and careful techniques in handling on the basis that preservation of delicate flavors also saves labile nutrients. The results justify the conclusion that food handling and processing need incur no such losses of vitamins as are often claimed. Data are given on the extent to which nutrients are retained and how abundantly natural foods supply dietary essentials.

While the food requirements of infants, invalids and the aged are different, they have much in common. It is acknowledged today to a far greater extent than a few decades ago, that a balance of dietary essentials, including the various minerals, the amino acids, and the numerous vitamins, plays a very wholesome role during invalidism and senescence as well as during infancy. Invalidism is constantly decreasing, thanks to medical progress and dietetic application of modern knowledge of nutrition, but by the same token, the aged are becoming more numerous.

The dietary requirements of these groups are not entirely satisfied by supplying nutritional essentials in satisfactory balance. It is required that the foods be flavorsome and palatable, preferably without spices and artificial flavors. Much can be accomplished in this respect by judicious combinations and mixtures. Some such combinations are well established and founded on good reason, while others are perpetuated by custom and tradition, two factors which unfortunately often rule out desirable dietary combinations.

Foods for these groups are required to be in suitable physical condition. This requires manipulation through various processes. Unless properly arranged and controlled they might seriously affect the food both from the standpoint of palatability and nutritive value. Prepared foods have been severely criticized at various times for loss of nutrients. Maynard *(4)* recently wrote: "If all the potatoes consumed in the United States in 1945 contained, as eaten, the vitamin C present when they were dug, over half the recommended allowance for our entire population would thus have been met. Unfortunately, most of it was lost before it got to the table." McCay *(5)* may be similarly quoted, "When we add together the twenty-five to fifty vitamins, fatty acids, inorganic elements, and amino acids that are recognized today as essential constituents of human foods, it is almost unbelievable that survival was possible, especially after men started to process and destroy natural foods." Another recognized authority stated that as much as 85 percent of certain dietary essentials often do not reach the consumer's plate.

The food technologist knows this need not be the case. He has at his command equipment which, if properly utilized, enables him to achieve ends otherwise impossible. It will be understood as axiomatic even without giving details, that it is better to avoid aeration than to resort to deaeration; that it is better to prevent the development of spoilage organisms than to establish processing times and temperatures to insure against all eventualities. The shortest period possible from the raw material to the sealed container is always a worthy aim. And, as the banal phrase puts it, the food technologist must also "know his onions." To illustrate, Irish potatoes and sweet potatoes on first thought might be expected to behave in a comparable manner with reference to the stability of their vitamin C content. As Table 1 shows they behave vastly different. Irish potatoes and sweet potatoes were each cooked, after

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being vacuumized and sealed in cans, in the absence of air in air-free water. They were then pulped in a Waring blendor for one-half minute with 2 to 2½ times their weight of water; and the vitamin C determined. The amount of pulp in the Waring blendor was then gauged to obtain a vortex of at least one-half inch in diameter when the blendor was run to secure thorough aeration. After each interval of running as indicated in Table 1, the vitamin C was again determined. Between each run 2 minutes were allowed for sampling.

Even after a total of 10 minutes of very thorough aeration plus 4 minutes of holding between intervals, there was still no loss of vitamin C in the sweet potato pulp. After 5 minutes more aeration and then holding for one hour the retention was still 85.4 percent as compared with 47.3 percent in Irish potatoes. These pulps become heated in the Waring blendor and the temperature at each sampling is given.

TABLE 1

Relative Stability of Ascorbic Acid in Sweet Potatoes and Irish Potatoes

Time in Running Waring Blendor	Sweet Potatoes		Irish Potatoes	
	Temp.	% Retention	Temp.	% Retention
½ minute	27° C.	100	27° C.	100
+ 2 minutes	31° C.	100	35° C.	99.5
+ 3 minutes	37° C.	100	45° C.	95.6
+ 5 minutes	45° C.	100	53° C.	90.8
+ 5 minutes	51° C.	97.2	58° C.	82.2
Held 1 hour		85.8		47.3

Certainly no food should ever be subjected to such oxidative conditions as are represented in the above experiment. Yet the losses are far less than indicated by the references cited above.

Practically all foods have some reducing effect on dehydroascorbic acid when heated with it. This effect will be illustrated below. While enzymes often produce deleterious effects they frequently may be employed to achieve wholesome advantages. Striking examples of the latter were recently reported by Kohman and Rugala *(1)*, whereby sugar for palatability as well as an increase in thiamine, as measured by the fermentometer, were obtained by the action of enzymes.

With these considerations in mind, the following five strained mixtures of foods have been prepared:

1. Beef with 6 vegetables and egg noodles.
2. Chicken with 5 vegetables and semolina.
3. Lamb with 5 vegetables and barley.
4. Beef liver with 7 vegetables and oatmeal.
5. Eight vegetables with oatmeal.

The following indicates how well these products supply dietary essentials. The Food and Nutrition Board of the National Research Council, (Reprint and Circular Series No. 116, January, 1943) does not specify the calories required for babies under a year of age, undoubtedly because it varies so much due to the large proportionate weight increment during the first year. But from one to three years, 1200 calories are specified. The Dietetic Analyses[b] of the five mixtures above permit calculating how well the dietary essentials (for which the Food and Nutrition Board

[b] Vitamin analyses of food mixtures by Vitamin Research Laboratory of Children's Hospital Philadelphia, Pa.; others by Campbell Soup Company Control Laboratories.

Calcium and Iron in milk according to Sherman, Chemistry of Food and Nutrition, The Macmillan Co., 1937; Niacin according to Holmes, et al., *J. Dairy Science* **27**, 849, 1944 and Hodson, *J. Nutr.* **29**, 137, 1945. Other vitamins in milk according to U. S. Dept. of Agr. Cir. No. 638.

has recommended amounts for babies 1 to 3 years old) are supplied by these five food mixtures if taken to provide 1200 calories and also if taken with milk in varying proportions to supply 1200 calories. Table 2 gives such data:

TABLE 2

Ability of Prepared Foods to Supply Recommended Dietary Essentials for Babies

Food Mixtures, 1200 Calories Supply	Food Mixtures, 865 Calories + 1 pt. Milk, 335 Calories Supply	Food Mixtures, 707 Calories + 1½ pt. Milk, 493 Calories Supply	Food Mixtures, 530 Calories + 1 qt. Milk, 670 Calories Supply	
1.7	1.6	1.6	1.5	Times recommended Protein
32.0	23.0	18.0	15.0	Times recommended Vitamin A
1.8	1.6	1.5	1.4	Times recommended Thiamine
2.9	2.3	2.1	1.8	Times recommended Ascorbic Acid
4.3	4.1	4.0	3.8	Times recommended Riboflavin
4.4	4.0	3.8	3.5	Times recommended Niacin
0.69	1.1	1.3	1.5	Times recommended Calcium
1.6	1.14	0.9	0.7	Times recommended Iron

The most important features of the above data are the ability of the milk to supplement the calcium value of the food mixtures, the ability of the mixtures, in turn, to supplement the low iron content of the milk, and the abundant vitamin A supplied by the food mixtures.

The abundance of vitamin A in the food mixtures is contributed by the liver in the one food mixture and the green and yellow vegetables in each. It is evident that with such food mixtures it is relatively easy to have an abundance of vitamin A and little of it is lost or destroyed in any ordinary food handling or cooking process. This is of added significance in view of Sherman's recent reports *(6)* on the advantages of an abundance of vitamin A in the diet. One quart of milk (670 calories) supplies 1760 I. U. vitamin A while 1200 calories of milk (1.79 qt.) supplies 3150 I. U. vitamin A or 1.6 times the recommended amount.

Another notable feature is that while the foods used are not highly regarded in the public mind as sources of vitamin C, in comparison with citrus fruits, yet 1200 calories of these mixtures supply 2.9 times the recommended amounts of vitamin C. Even when a quart of milk replaces its calorie equivalent of these food mixtures, 1200 calories still supply 1.8 times the recommended amount. Few realize the high vitamin C content of many of our vegetables and of liver.

The low calcium figure in the food mixtures is not surprising since it is known that generous amounts for growth are difficult to obtain without milk. The calcium figure could be raised easily with green leaf vegetables but at the sacrifice of flavor. For babies, this low calcium figure is of little consequence since such foods are intended to be used with milk, a pint of which provides the full requirement of calcium as is evident from the second column in the above table.

For adults, invalids and the aged, these foods supply ample calcium, as is apparent from the following consideration. The Food and Nutrition Board of the National Research Council recommends 2500 calories for sedentary males and 2100 for females, or a median of 2300. If these five foods are taken in amounts to supply 2300 calories, they will supply:

2.0 times the recommended Protein
24.8 times the recommended Vitamin A
1.6 times the recommended Vitamin B-1
2.7 times the recommended Vitamin C

3.8 times the recommended Riboflavin
3.8 times the recommended Niacin
1.7 times the recommended Calcium
1.8 times the recommended Iron

In the federal government's efforts in nutritional education the idea of the "basic seven" food groups is emphasized. In developing these five mixtures, group 4, milk and milk products, was purposely excluded because the food mixtures were intended as supplements to, not substitutes for, milk, which is nearly always readily available in suitable form. Group 7, butter, margarine and cod liver oil, was also excluded because in foods for the groups under consideration, the fat content should be relatively low. The fat in these mixtures of natural foods furnishes 20 percent of the calories. Moreover, the vitamin A supplied by butter and margarine is amply supplied without them and while the vitamin D of cod liver oil is not generally expected from ordinary foods, it was previously shown by Kohman, Sanborn, Eddy and Gurin *(2)* that food mixtures of this type stimulate calcium assimilation when added to the severely rachitogenic Steenbock diet, similar to cod liver oil. Although tomatoes were used, it was not to meet the requirement of group 2. Yet, as shown above, the main dietary essential of this group, vitamin C, is well provided in these mixtures. Table 3 indicates how well the other groups are provided.

It is evident that these foods supply the dietary essentials in question in abundance. This permits a low calorie intake without sacrificing essential nutrients.

TABLE 3

Representation of U. S. Basic Food Groups in Food Mixtures

U. S. Seven Basic Food Groups	Individual Foods Provided	Food Mixture * 1	2	3	4	5
Group 1 Green and yellow vegetables	Carrots	×	×	×	×	×
	Sweet Potatoes	×	×	×	×	×
	Peas				×	×
	Okra					×
Group 2 Oranges, Grapefruit, Tomatoes, Salad Greens	Tomato	×			×	×
	Parsley		×			
Group 3 Potatoes and other vegetables and fruits	Potatoes	×		×	×	×
	Celery	×	×	×	×	×
	Onion	×	×	×	×	×
Group 5 Meat, Poultry and Eggs	Beef	×				
	Liver				×	
	Lamb			×		
	Chicken		×			
	Egg	×				
Group 6 Bread, Flour and Cereals	Noodles	×				
	Oatmeal				×	×
	Barley			×		
	Semolina		×			

* Food Mixtures:

1. Beef with 6 vegetables and egg noodles.
2. Chicken with 5 vegetables and semolina.
3. Lamb with 5 vegetables and barley.
4. Beef Liver with 7 vegetables and oatmeal.
5. Eight vegetables with oatmeal.

Stare *(7)* recently stated: "Excessive calorie intake is the single most extensive nutritional problem affecting public health in this country." The following data indicate to what extent the high vitamin content is due to efficient conservation of the amounts originally present in the individual products used. The vitamin C values of the raw products are given in Table 4.

TABLE 4

Ascorbic Acid Content of Raw Ingredients

	mg./100 grams
Carrots	7.6
Sweet potatoes	19.6
Potatoes	13.9
Peas—frozen	14.3
Onions	7.6
Celery	8.4
Okra (salted and cold storage)	7.5
Tomato Paste (26% solids)	84.7
Parsley	205.0
Liver	36.2
Lean Beef	2.0

It is physically impractical to make all these determinations on the exact lots of material that go into the particular batch of mixture on which the vitamin C of the finished product is to be determined. These values, based on several determinations, fall well within the range given in the 'Compilation of Vitamin Values" in the U. S. Dept. of Agriculture Circular No. 638.

The figures in Table 5 are self explanatory:

TABLE 5

Ascorbic Acid Retention During Preparation and Processing

Mixture No.	mg./100 grams				
	1	2	3	4	5
Calculated from raw products	5.68	1.87	4.52	10.50	11.10
Determined when filled into jars	5.48	1.34	3.31	7.61	8.63
Determined after processing and cooling	6.18	1.69	4.01	7.94	9.05
Percent retained	109	90	89	75	81

It is to be noted that the vitamin C values increase during processing for sterilization. In the first cooking of any food, there is always oxidation of some of the ascorbic acid to dehydroascorbic acid, largely in the beginning of the cooking period. Unpublished data show that subsequent heating, after all oxygen has been dissipated, causes certain reducing substances to reduce part of the dehydroascorbic acid back to ascorbic acid. This is to be expected since such foods give off hydrogen sulfide which is used in an analytic procedure for the reduction of dehydroascorbic acid to ascorbic acid; also, they contain glutathione which has previously been shown by Kohman and Sanborn *(3)* to reduce dehydroascorbic acid.

Wagner, Strong and Elvehjem *(8)* recently reported that heat processing increases the capacity of corn to reduce the indophenol dye but concluded that it is questionable whether the increases are due to ascorbic acid. This is not an uncommon phenomenon. That this increase probably does represent ascorbic acid was shown by adding iodine-oxidized ascorbic acid to these five food mixtures after they had already been processed once and stored for over a year, then reprocessing them. Since the iodine was dissolved in potassium iodide solution, and since when

iodine oxidizes ascorbic acid a stoichiometric amount of hydriodic acid is formed, as controls the same amount of potassum iodide and hydriodic acid were added to adjacent glass jars of the food mixtures. The following increases calculated as milligrams ascorbic acid per 100 grams from the dye titration due to the added dehydroascorbic, were obtained:

TABLE 6

Increase in Titration Value During Processing in Presence of Dehydroascorbic Acid

Mixture No.	mg./100 grams				
	1	2	3	4	5
Without dehydroascorbic acid	2.55	0.75	0.90	6.00	3.90
Added dehydroascorbic acid	7.30	2.30	4.65	11.00	7.60

The low values in the mixtures without added dehydroascorbic acid are due in part to dilution in making the addition of the KI-HI mixture, also due to previous storage and to the usual normal loss.

There is considerable variation in the percent of vitamin C conservation in the five mixtures, the figure for No. 1 being an impossible 109 percent. Such a result is not uncommon and is most likely explained by the fact that the raw products used vary over quite a wide range from day to day and between lots of the same day and the exact raw products that go into a given finished batch cannot all be analyzed because of physical limitations. By the same token the 75 percent retention for mixture No. 4 is probably too low. The average of the five figures is 89 percent and it should more fairly represent an over all percent of conservation.

Destruction of vitamin B-1 in cooking foods is not a matter of oxidation as with vitamin C but the direct effect of heat. It was possible in preparing these mixtures to sample large batches in a homogeneous condition after no more than 15 to 20 minutes of cooking at atmospheric pressure. The thiamine was determined at this point and again after processing—sterilization at 240° F. Since the percent loss during this longer period at a far more severe temperature was moderate, it is evident that the loss in the preliminary precooking period must have been negligible. Unpublished data by Kohman and Rugala *(1)* show that such brief cooking increases the available thiamine in some products, as measured by the fermentometer. From the figures given below it is evident that the percent loss increases with decreasing thiamine content. This is probably not a case of cause and effect. It so happens that the pH in these mixtures varies inversely with the thiamine content and the time of processing must be made to vary with the pH. Increase in pH and processing time and temperature are the real factors that are responsible for higher thiamine loss.

TABLE 7

Thiamine—mg./100 gms.

Mixture No.	1	2	3	4	5
Unprocessed	.039	.029	.035	.070	.067
Regular Process	.029	.016	.023	.062	.060
Loss	26%	45%	34%	11%	10%
Loss during 10 min. Added to Process	10%	....	28%	14%	....

The ten minutes increase in process is well within the range of what becomes necessary if spores of spoilage organisms in the raw product are tolerated or if they are permitted to develop anywhere along the line.

CONCLUSION

There is no denying that certain dietary essentials may be destroyed to a serious extent by poorly controlled food preparation processes. It has been shown herein that complicated processes, necessary to prepare foods to meet exacting physical requirements and to sterilize them for distribution ready to serve, can be controlled to reduce loss of nutritive values to almost negligible quantities. Thus, they supply in abundance the minerals that often are extracted and discarded, and vitamins that may be either extracted or destroyed. By judicious choice in making up mixtures, flavor and palatability may be attained in natural foods alone without spice or condiments.

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EVALUATION OF FOAM-PRODUCING AGENTS[a]

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REVIEW PAPER

Discussed are foams and whipping agents in relation to the manufacture of certain kinds of foods and the production of foaming or whipping agents from the soy bean.

By far the largest part of the food which man consumes is in colloidal form. This applies to the natural foods such as meat, milk, fruit, vegetables, etc., as well as to manufactured foods, like butter, bread, ice cream, cereal foods and candy. Indeed, there are but a very few foods which are not a disperse system of some sort.

Excluding systems in which gas is the dispersing medium, there are three colloidal systems in which a solid is the dispersing medium and three in which a liquid is the dispersing medium. Pertinent to this discussion is the system in which a liquid is the dispersing medium while a gas, usually air, is the disperse phase. Foods of the gas-in-liquid system, or foams, are the least important from the standpoint of variety as well as quantity. They are necessary, however, in the production of many foods because they impart to them proper eating characteristics.

Foams in foods (Table 1) represent three types, viz.:

1. A manufactured foam, sold and consumed as such. An example, marshmallow candy.

TABLE 1

Classification of Foams

Name	Use	Whipping Agent	Whip Based on		Properties of Foam	
			Total	Sugar	Water	Weight
			%	%	%	lbs./gal.
Mazetta	Candy	Egg albumen or soya	1.0	1.2	16-18	7-8
Frappé	Candy	Egg albumen or soya	1.75	2.25	20-24	4-5
Marshmallow	Candy, Biscuits	Gelatin, or gelatin and soya (3 + 1)	2.0	2.9	28-30	3.0
Meringue	Pies	Egg albumen and gelatin	1.8	3.5	40-45	1.5

2. A manufactured foam used as an intermediate in food manufacture. A candy mazetta is of this type.

3. A potential foam, which is created voluntarily or involuntarily by the consumer. Whipping cream and beer belong in this class.

Of particular interest at the moment is the class of foams made by whipping or beating some form of syrup with a so-called foaming agent to produce a stable foam of certain desired characteristics. The mixture must be fat-free in order to whip up properly. These foams may be classified on the basis of moisture content, density or pounds per gallon, intended use, or the whipping agent used to produce and hold the foam.

SOYBEAN WHIPPING AGENTS

A recent development in this field is the production of foaming or whipping agents from the soybean. In general, these are made as follows:

The base material is a soybean flake or grit from which the oil has been extracted.

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The residual oil in the flake should be less than one percent, and extraction and solvent-removal should be carried out at a temperature low enough to prevent any denaturation of the protein.

The oil-free flakes are extracted with water. The water extract contains a little over 50 percent of protein on the dry basis; the balance of the solids are minerals and a variety of soluble carbohydrates such as di- and trisaccharides and soluble gums.

When the water extract is brought to the iso-electric point, most of the soybean globulin is precipitated. The albumen remains in solution. The supernatant liquid is drained from the precipitated protein which is washed by decantation.

The protein in this form has little or no whipping properties. The ability to whip is produced by partial hydrolysis, and there are several methods by which the hydrolysis may be carried out successfully. The details of the hydrolysis are trade secrets of the various manufacturers.

The partially hydrolyzed protein is then dried in a conventional spray drier. Because it is very hydroscopic it must be packaged in a moisture-proof container.

The method of manufacture as just described seems simple enough, but in commercial practice it presents some tough problems. The soybean contains no starch. Its carbohydrates are about half-soluble and half-insoluble in water. Certain of the carbohydrates swell in water to form a viscous suspension, which makes extraction of the protein, filtration of the extract and precipitation of the protein difficult in any except very dilute solutions. Some day these vegetable gums will no doubt be separated and dried to form useful substitutes for pectin, agar, gum arabic, and similar products, but in the large-scale preparation of soya protein they cause serious difficulties.

PROPERTIES OF THE WHIPPING AGENTS

Various materials have been used for producing foams high in sugar solids. Since each of these whips in a different manner and produces foams of widely varying characteristics, a description of their properties may be of interest (Table 2).

TABLE 2

Composition of Whipping Agents

	Soya	Gelatin	Egg Albumen
	%	%	%
Moisture	5.0	12.0	10.0
Protein (N × 6.25)	65.0	87.0	80.0
Ash	8.0	1.2	4.5
Carbohydrates, etc.	22.0	9.8	5.5
Water-soluble protein	69-95	100	100

Saponin. At one time some form of the soap-wort plant was used extensively for producing foams of all sorts. Saponin is quite toxic, however, and its use is illegal in foodstuffs.

Egg albumen, fresh, frozen or dried. Egg albumen is probably the most widely used of all the foam-producing agents. While not the best for each purpose, it has at times been used for all of the foams under discussion. It is the most versatile of all of the foam-producers, since it will whip well in foams of all types, whether low or high in moisture, or when used with boiling or cold syrups. Morever, it holds about twenty times its weight in water, which prevents separation of syrup after the foam is made. It holds this water either when beaten in the cold or when coagulated by adding boiling syrup to the albumen solution at the beginning of the whipping operation.

Egg albumen foams of high moisture content lack body and elasticity, and hence albumen is seldom used now in marshmallow. It is ideal for meringue powders, where high moisture, large volume and a short, tender foam are desired.

Gelatin. Gelatins vary widely in their characteristics and in their utility for various purposes. Gelatin is commonly manufactured from three raw materials, viz.:

(a) Pig-skin. The trimmings from pig-skins are probably the largest source of gelatin manufactured in the United States today. Pigskin gelatin is of excellent quality for most uses, although some from this source tends to be too high in viscosity for optimum whipping characteristics.

(b) Calf-skin gelatin is made from hide trimmings. It shows good qualities as a whipping agent, and when available it is commonly used in blends of gelatins for marshmallow.

(c) Bone or ossein. Ossein is an intermediate product made from bones. Before the war the domestic manufacturer of gelatin purchased ossein from Europe and converted it to gelatin. The shortage of ossein was one of the chief causes of the gelatin shortage during the war, and the scarcity still persists. Ossein gelatin has excellent characteristics for whipping syrups into foams, since it does not have too high a viscosity.

The market value of gelatin depends largely on an empirical figure called the bloom test or bloom strength. Bloom is a measure of the gel strength of a gelatin. A gel is made by dissolving gelatin in water, cooling, and testing the resistance of the gel to penetration under carefully controlled conditions. Bloom strengths of gelatin range from about 100, which is too low for most edible uses, up to 300, which is very high for satisfactory whipping. For marshmallow, bloom strengths of 175 to 250 are commonly used. The lower strengths, 175 to 200, are preferable for marshmallow work in the opinion of many, although all strengths are used very satisfactorily.

Another test of importance for gelatin is the viscosity of a solution in water. Gelatin for whipping use should not have a high viscosity in relation to its bloom strength, since too high a viscosity will reduce or even prevent the ability to whip.

Since no two lots of gelatin are identical, and because a single gelatin is seldom of optimum quality for a specific use, most gelatins offered today are blends. These blends are made to obtain the correct bloom as well as to possess certain intangible characteristics based on its intended use.

As a whipping agent gelatin has certain limitations. It will not whip up well if the sugar-syrup-gelatin mixture has less than 28 percent total moisture. If the glucose, or corn syrup unmixed, constitutes 40 percent or more of the sugar solids in biscuit marshmallow, the volume is low and the marshmallow is "runny" and does not set up to a stiff foam. In addition to marshmallow, gelatin is also used with egg albumen in meringues and meringue powders. Albumen alone makes a tender meringue of high quality, but the cost is high and the meringue will not stand much shipping, nor will it stand up for long periods of time. Gelatin helps to overcome these deficiencies.

(d) Soya Whipping agents. The manufacture of these has been described. As would be expected, they differ from the other whipping agents in several respects. Soya whipping agents whip more rapidly and to greater volume than egg albumen or gelatin. Properly made, they will do the most difficult beating job of all, which is to make candy mazettas and frappes. In general, the lower the moisture content of a foam, the more difficult it is to whip it to full volume, and mazetta is the lowest in moisture content of the foams under consideration.

Soya whipping agents, like egg albumen, do not give enough body to marshmallow when used as the sole whipping agent. They are useful as a partial replacement of gelatin (up to 25 percent) to increase the speed of the whipping and to make and keep the marshmallow tender. Strong gelatins tend to make tough marshmallow. This tendency towards toughness can be reduced to any desired point by replacing part of the gelatin by a soya whipping agent. Soya whipping agents have one deficiency as compared to egg albumen in that they do not coagulate with heat. For this reason, they do not hold water as firmly as egg albumen. In low-moisture foams, where the body and water-holding capacity depend chiefly upon the character of the dispersion and size of the foam cells, this lack of coagulation is not serious. In light foams of high moisture content, such as meringue powder, the lack of coagulating ability may cause them to give unsatisfactory results.

A short description of the various foams may be of interest.

Mazetta. This is a very stiff, heavy foam which is not easily broken down and when made as described below will have a moisture content of about 18 percent. Most other foaming agents, including gelatin, will not work with such a low moisture content. Either egg albumen or soya can be used in its preparation. It is added to cream centers, fudge, and other candy mixes in order to introduce air into the mixes and to control the crystallization of the sugar. It is interesting to note that nougat is substantially a mazetta to which has been added about five percent of fat.

It is made by pouring a hot syrup over a foam in a beater in motion. The syrup, boiled to 240° F. (116° C.), consists of glucose 40 lbs., sugar 10 lbs., and water 8 lbs.; the foam already in the beater consists of glucose (CSU) 50 lbs., either egg albumen or soya whipping agents 1.5 lbs., and water 3 lbs.

Frappe or Marshmallow Topping. A frappe is similar to a mazetta, except that more whipping agent is used, the moisture content is higher, and invert syrup may be added to increase the hygroscopicity of the finished candy. It is used in various candy mixes such as caramel, kisses, fudge, cream centers and nougat for the purpose of adding air to the batch and to control sugar crystallization. Again, either egg albumen or soya whipping agent is used. A frappe has a moisture content of about 22 percent. It is a stiff, heavy foam, but has less body and greater volume than a mazetta.

Biscuit and Candy Marshmallow. These are basically similar products, in that they contain two to 2.5 percent gelatin, 28 to 32 percent moisture, and 66 to 70 percent sugar solids. Differences in marshmallow are due largely to variations in the sugars. The sugar solids can consist of mixtures of sugar, corn sugar, corn syrup, invert syrup, malt syrup, molasses and various other syrups, with suitable limitations based on flavor. Not over 40 percent of corn syrup (glucose) can be used or the mixture will not beat up to the desired volume and consistency.

Marshmallow should be firm but tender, elastic and springy, with a good body. Gelatin is the only whipping agent which will give marshmallow the desired physical characteristics. To lessen the toughness of marshmallow, up to 25 percent of the gelatin may be replaced with excellent results by a soya whipping agent.

Meringues and Meringue Powders. Originally a pie meringue was made by adding sugar to fresh egg whites and beating the whole to a stiff foam. The foam is placed on a pie and browned in the oven. This is still one of the best means for making high quality meringues, and is the method used in the household, by restaurants and hotels, and even by some commercial pie bakeries.

For commercial meringues which must stand abuse in shipping and storage, it is customary to stabilize the meringue by using gums and a small amount of alum, and by adding gelatin. Mixtures of egg albumen, gelatin, gums, starch and alum

are called meringue powders. Soya whipping agent may replace part of the albumen in a meringue or meringue powder. The total of whipping agent in these ranges from 25 to 50 percent of the meringue powder. In use, five ounces of meringue powder, one quart of water and 2.5 pounds of sugar are whipped to the desired volume.

A meringue has the highest moisture content of all the foams, from 40 to 45 percent. It is the highest in specific volume. It should be close-textured and tender, and should not lose water in spite of its high moisture content.

Several interesting facts are pertinent to the foams discussed above, to-wit:

1. Low-moisture foams can not be made with all types of whipping agents. They can not be whipped to a high specific volume with any whipping agent.

2. As the moisture content of the foam increases, the ease of whipping increases in proportion, and the foam is much higher in specific volume.

3. Each whipping agent shows specific characteristics. The character of the foam depends on the type of whipping agent used, the moisture content of the foam, and the types of sugar or sugars. Whether differences in foam characteristics due to the whipping agent are dependent solely on foam structure and cell size or to the specific nature of the whipping agent is not definitely known. Both factors probably play a part.

4. Modifying the character of the foam by adding small amounts of other materials is usually not very successful. For instance, a marshmallow made solely with egg albumen or soya whipping agent has almost no body and no elasticity or springiness. Adding gums, alginates, modified starches and other similar materials does not increase the body or elasticity. The desired physical characteristics can be obtained only when gelatin is used as the principal whipping agent.

5. The protein whipping agents recently derived from the soybean have opened up a new field in the production of foam-producing materials. These materials have become very useful in the candy and biscuit industries in producing stable foams. Undoubtedly there will be further developments in this field, with the ultimate production of a series of foam-producing agents to fit any possible need.

POSSIBILITIES IN DEVELOPING FISHERIES BY-PRODUCTS[a]

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REVIEW PAPER

The author reviews some of the phases of fishery by-products in the light of the more recent work, particularly fish meal, recovery and uses of "stick-water," proteins, fish wastes and possibilities of obtaining biological products from these wastes.

It will soon be twenty-five years since D. K. Tressler published, in collaboration with others, a book which I think has proven invaluable to most fishery technologists *(98)*. This book was entitled "Marine Products of Commerce," and a new edition has recently been published. It seems unfortunate that this book has not been revised in order to make it thoroughly up-to-date, though there is no doubt that some of the sections could pass with little revision. In this work, a number of chapters are devoted to by-products of the world's fisheries, and a perusal of these is sufficient to assure one that a vast variety of such products exists. In the time at my disposal it will be possible to cover only some of the phases of fishery by-products research, and in most instances no attempt will be made to state whether commercial production of any of the various products discussed is likely to prove profitable.

In considering the term "by-product" as related to the fishing industry I have avoided, with a few minor exceptions, reference to any item which is intended for consumption as a nutritious human food. In fact this would perhaps be a useful definition of the above term, since it would automatically exclude such items as fish sausages, loaves, or canned fish trimmings, but could include agar, alginic acid and vitamin oils, which, though consumed, can hardly be considered nutritious foods. It is almost impossible to divide by-products into very strict classes since they are of such varied nature, so I have only attempted to classify them into rough groups. Other reviews dealing with certain by-products are available *(59, 60, 98)*.

BY-PRODUCTS OF A PROTEIN NATURE

The days when, as one fish foreman stated, it was possible to "sell anything with a fin on it," seem to be drawing to a close. Meat and other animal protein foods appear to be in more plentiful supply, and it is inevitable that under these conditions a surplus of fish may develop. Indeed, even at the present time, the market for certain of the inferior grades of white fish is becoming restricted on this continent. It is hoped that some of the surplus may be utilized by canning, and a fairly palatable canned product can be prepared if a method which avoids accumulation of surplus liquor in the can, and brown discoloration of the fish is followed *(19)*. However, there is much loss in canning certain types of white fish, and there may be only a twenty percent yield of edible fish in some cases. This inevitably makes cost of production quite high and the product must compete with tasty canned fish such as the higher grades of salmon, sardines, etc. I think then, that the most important single problem which will require solution is that of disposal of surplus first class fish protein at a price which is profitable to fishermen and to the industry. There is plenty of room for research in this field.

The chief protein by-product of the fishing industry is fish meal which is normally produced from so-called "scrap fish," fish offal and shell fish wastes. The general methods for commercial production of fish meal are quite well-known and will not be discussed *(21, 59)*. One west coast firm is drying the cooked flesh by

[a] Presented before the Seventh Annual Food Conference, Boston, Mass., June 4, 1947.

passing hot air through a thin layer of the comminuted material in order to avoid undue heating of the product which is liable to occur by ordinary drying methods. Fish meals, especially those containing fish liver and "solubles" *(11, 57)*, are usually in great demand for addition to livestock rations, for they generally contain adequate concentrations of the essential amino acids *(1)*. However, the raw material used in their preparation may occasionally contain much poorly digestible cartilaginous material, or perhaps urea, and it has been suggested that in using meals some cognizance should be taken of their content of amino acids essential for livestock feeding rather than simply referring to their nitrogen content. Present methods of determining amino acids are rather time consuming and exacting so it is difficult to decide whether or not the above suggestion is feasible. At our Station a study of the essential amino acid content of certain fishery products is being made using microbiological assay methods *(29)*, and it is intended to extend this work to include a survey of different fish meals and to eventually attempt the isolation of amino acids from fish proteins. High quality fish meal has been said to be one of the few protein sources which will support chick growth entirely by itself *(1)*. Recently it has been established that it contains a "Factor S" which is apparently essential for growing chicks, and that this factor appears to be identical with the Strepogenin recently discovered by Woolley at the Rockefeller Institute *(89)*.

Fish meal is normally prepared by cooking the flesh and pressing it. The cloudy press liquid which results after removing the greater part of the oil by centrifuging, or by use of settling tanks, still contains suspended and soluble proteinaceous material, and some oil. This liquid is commonly known as "stickwater" or fish press liquor, and it has been realized for some time that much valuable nitrogenous material is wasted when this is discarded *(7, 29)*, and that it is liable to cause undesirable pollution of rivers or harbors if it is discarded *(56)*. Attempts have been made to recover the proteinaceous material from these waste liquors *(7, 50)*, but it is apparently only quite recently that recovery has been realized commercially, and even now large quantities are wasted annually in British Columbia. The liquor may be treated in two different ways for incorporation in livestock feeds. At present the best procedure appears to be that of multiple effect evaporation, so that a concentrate containing about 50 percent of solid matter is obtained. This thick liquid can be added directly back to the comminuted cooked flesh before it is dried into meal, incorporated in dry kelp *(Macrocystis)* meal for livestock feeding or may even be shipped in tank cars and incorporated in feeds at its destination. Alternatively it may be fractionated as follows (56): It is first adjusted to pH 4 to 5 with an acid protein coagulant such as sulfuric or tannic acid. This breaks the emulsion and when the liquor is centrifuged, from 0.5 to 2.0 percent of oil is recovered and the oil alone is said to be of sufficient value to pay for the whole treatment procedure. The protein solids may be filtered off; they account for about 2 to 3 percent of the liquor and may be dried with the fish meal. The clear, sparkling filtrate contains soluble protein material and the B vitamins, thiamine and riboflavin. It may be concentrated by evaporation, or, at its low pH value the B vitamins may be adsorbed on fuller's earth which is added in proportions of 0.5 to 1 percent. The fuller's earth, which contains the adsorbed vitamins, can be dried, after removal by centrifugation, and added directly to livestock feeds. Another suggested method for separation of protein solids from stickwater involves saturation of the hot liquor with a salt such as sodium chloride or ammonium chloride, which causes physical flotation of the small particles, cooling and mechanically removing the jellied supernatant layer *(62)*. This method does not appear very practical for it would involve use of enormous quantities of salts. It is said that in Norway, stickwater is mixed with the partially dried meal, so that the meal after final drying is richer in B vitamins and soluble

extractives. An idea of the potential value of stickwater to the fishing industry can be obtained from figures which have been published *(103)* regarding the sardine or pilchard industry. The estimated annual production in 1945 was, in millions of pounds: canned fish, 150; fish meal, 155; oil, 104 and stickwater, 7. The recent poor pilchard runs would seem to make it even more imperative that stickwater be recovered.

The "condensed fish solubles" obtained by concentrating stickwater have valuable nutritive properties *(11, 27)*. When fed at 12 percent level they were found to produce excellent growth in chickens, but above this level exerted a cathartic action. When both diets were supplemented with riboflavin the press water concentrate was as effective as skim milk in chick nutrition *(57)*.

The value of fish wastes as fertilizers is well-known. In this connection it is interesting to note that incorporation of stickwater into peat moss, which can be subsequently dried, results in an excellent fertilizer which will increase the humus content of poor soils *(62)*. One west coast firm has evolved a method for extracting, from salmon offal or from fatty scrap fish, oils which have a considerably higher vitamin A potency than that normally occurring in oils extracted by usual commercial procedures. The protein residue, which is quite alkaline in reaction, contains large amounts of inorganic salts, and consequently cannot very well be incorporated in livestock rations. However, it has been found to possess valuable properties as a fertilizer. Whether or not it is desirable to increase the yield of vitamin A in the extracted oil at the apparent expense of first-class feeding protein would seem questionable.

It is well-known that certain fish wastes, particularly those containing large amounts of collagen (skin and heads), can be manufactured into glue *(98)*. Fish glue usually contains significant quantities of proteins and peptones and is therefore inclined to be soluble in cold water *(59)*. Dialysis of stickwater to remove salts, and subsequent evaporation, has been found to form a useful method for preparing fish glue *(92, 93)*.

Synthetic egg white prepared from fish protein has been used for some years in Germany *(5, 15, 31, 100)*. The general method of preparation is, apparently, to remove the readily soluble fish flesh protein with dilute acetic acid, and extract fat from the residue with trichlorethylene or ethanol. The extracted residual protein is stirred with warm dilute sodium hydroxide solution. The partially hydrolyzed extracted protein thus obtained is presumably largely myosin. It is neutralized with dilute acetic acid and spray dried to yield a white powder containing 94 percent protein. Its amino acid composition suggests that it would have a high nutritive value.

Fish scales are a source of useful by-products. The scales of several species of fish yield small lustrous crystals of the purine guanine, and these form the pearl essence of commerce *(24, 98)*, which is used to coat the surface of artificial pearls. However, other possibilities have been suggested in the utilization of fish scales. Guanine itself can be made from the scales. It is said that caffein, which chemically is rather closely related to guanine, has been made in considerable quantities from fish scales *(4)*. It is probable that the scales are analogous to human hair in the fact that they apparently contain considerable quantities of cystine; one company is said to use scales as a source of raw material for manufacturing this amino acid.

Certain of the internal organs of fish offer possibilities as sources of hormones, enzymes or other interesting and useful by-products. In 1901 it was shown by Rennie in Scotland that fish contain quite prominent islets of Langerhans, and he thought that these might prove of value in counteracting diabetes. Years later MacLeod, in whose Toronto laboratory Banting, Best and Collip succeeded in

preparing clinically administrable insulin, became interested in the occurrence of insulin in fish. A research program was initiated at the St. Andrews Station of the, then, Biological Board of Canada, and it was found that the principal islet of Langerhans in bony fish is usually attached to the gall bladder, from which it can be separated quite readily. Clinically active insulin, indistinguishable from that prepared from beef pancreas, was extracted from these islets *(65, 66)*. It is difficult to determine whether much insulin has been prepared commercially from fish, though it is said that the Japanese have developed this source. The demand for insulin is increasing so that there are strong possibilities that all available bovine pancreatic tissue may be required for its preparation *(13)*. If some of the commercial tryptic enzymes were prepared from fish viscera *(vide infra)*, valuable animal pancreatic tissue could be reserved entirely for insulin production. Another pharmaceutical product which has been prepared from fish is the anti-pernicious anaemia factor. Preparation was effected by making a dilute aqueous extract of haddock, cod or whiting livers (pH 4.5), heating it to 158° F. (70° C.), settling, filtering, concentrating in vacuo, precipitating the active principle with 95 percent alcohol and drying to a powder *(61)*. The anti-pernicious anaemia factor so prepared was found to be quite effective in a number of clinical trials *(28)*.

It will be recalled that the classical work of Meischer on the composition of sperm nucleoproteins was carried out over half a century ago, the raw material employed being ripe herring milt. The best known sperm nucleoproteins are clupein, from herring milt, and salmine from salmon milt. Chemically, sperm nucleoproteins consist of nucleic acid and sperm protein, or protamine, which are apparently quite loosely combined, as work by Pollister and Mirsky *(77)* at the Rockefeller Institute has shown. Of chief interest is the fact that these protamines consist of 15 amino acid residues, 10 of which are accounted for as the basic amino acid arginine, and the remainder probably as proline, valine, serine and alanine. Fish milts have been utilized to some extent commercially. Protamine is used to make protamine zinc insulin, which is practically insoluble and when injected liberates insulin slowly, giving a regulated action. Protamine from certain species of Pacific salmon has been employed for this purpose *(2)*. The occurrence of large amounts of arginine in sperm proteins makes the ripe milt of fish a very convenient source of this amino acid. Salmine has been found to reduce the clotting time of blood plasma *(12)*. It has also been found that clupein *(64)*, salmine *(70)*, protamine sulfate from shad *(18)* and protamine zinc insulin *(18)* possess marked antibacterial activity.

One step in the preparation of hide leather involves a softening which is brought about by a slight hydrolysis of collagen and elastin by crude proteolytic enzyme preparations; so-called "leather bates." It has been found that the pyloric caeca of fish (finger-like blind sacs which are attached to the gut just below the stomach, and have a function similar to that of the mammalian pancreas) are a potent source of tryptic enzymes. Crude enzyme preparations made by acetone drying of comminuted caeca were found to be just as effective as hog pancreas or commercial leather bates for leather treatment and for hydrolysis of isolated collagen, elastin and olive oil *(47, 48, 49)*. Recently, finely minced pyloric ceaca have been employed to prepare protein hydrolysates from fish flesh and from fish muscle myosin, and the enzyme preparations used effected about the same degree of hydrolysis as did commercial powdered pancreatin *(29)*. Crystalline salmon pepsin has been prepared from salmon stomachs *(73, 74)* and its specificity has been studied *(33)*. Properties of crude tryptic enzymes from fish viscera have been investigated *(51)*. In our own laboratories attempts are being made to prepare crystalline trypsin-like enzymes from the pyloric caeca of fish.

One suggested outlet for surplus fish flesh protein is that of preparation of plastics of the type which have been commercially made from casein: so-called "artificial horn." There are a number of possible methods whereby such plastics can be prepared. One method which has given good results in the laboratory is to dry comminuted fish flesh with alcohol or acetone at ordinary temperatures. The dry, powdered meal is mixed with 20 percent of ethyl lactate and pressed at about 12,000 lbs. per sq. in. and 203° F. (95° C.), or 10,000 lbs. per sq. in. and 221-239° F. (105-115° C.), for approximately 10 hours. The pressed plastic is then treated for at least 2 weeks in commercial formalin, which hardens it and prevents it from becoming brittle *(45, 46, 52)*. Recently it has been claimed that large sheets of plastic material have been prepared from fish flesh *(4)*.

There are other possible uses for fish flesh proteins. Protein hydrolysates prepared from casein, corn and wheat glutens have been found to be useful as flavoring ingredients for foods *(34)*. Protein hydrolysates are readily prepared from fish muscle, and, superficially at least, do not seem very different from those obtained from meat. This is understandable for, as far as is known, the muscle proteins myosin, myogen, globulin X, collagen and elastin are present in roughly similar proportions in both kinds of muscle *(6, 79)*, though the content in soluble extractives differs *(80)*. Probably it would be of interest to compare hydolysates prepared by tryptic or hydrochloric acid digestion of fish muscle and of cereal proteins with the object of ascertaining whether the former might be of value for addition to foods. In this connection it is of interest that one patent records the preparation of concentrates similar to meat extracts from meat of sea mammals such as whales and seals *(75)*. Possibly, similar extracts could be prepared from fish flesh. Removal of the slight residual "fish" flavor which tends to remain in fish protein preparations intended for use in human foods is said to present rather a difficult problem.

Peptones have been defined as the dried product of the pancreatic digestion of meat *(68)*. They have some commercial use, chiefly in preparation of microbiological media, and it seems reasonable to assume that it would be quite simple, and perhaps cheaper, to prepare them from fish muscle as raw material instead of from meat. A "peptone" has been made by hydrolysis of fish flesh with hydrochloric acid *(102)* and in our laboratories peptones have been prepared by hydrolyzing fish flesh with proteolytic enzymes obtained from fish. In recent years considerable interest has been taken in the so-called "food yeast," *Torulopsis utilis*. This yeast is rich in B vitamins and is comparable to casein in supplementing cereal proteins in a diet since it contains the much needed amino acids lysine and valine. It is somewhat deficient in methionine *(76)*. *T. utilis* grows well on peanut waste liquors containing an added ammonium salt *(55)*, and it seems possible that stickwater might find some similar use as a culture medium. It has apparently been employed in Japan in media used for preparing penicillin.

FATS, FAT SOLUBLE VITAMINS, STEROLS AND PHOSPHOLIPIDS

During the past decade there has been published a very large body of information regarding marine animal fats or oils, their occurrence, characteristics and uses, and much of this has been reviewed *(20, 59, 60)*. The use of hydrogenated marine oils in shortenings, cooking fats and in margarine manufacture is well-known. Fish oils are used and offer possibilities in the paint and varnish industries *(63)*. Other known uses for fish oils include incorporation in linoleums, oilcloths, patent leather, oiled silks, core oils, printing inks, rubber, synthetic detergents. etc.

Fish oils are still the principal source of vitamin A *(85)*, and certain oils such as those of halibut, red cod, ling cod, etc., can be used directly to supply this vitamin clinically. However, the supply of high vitamin A potency oils is usually rather

restricted and considerable effort has been directed toward concentrating vitamin A from fish oils of moderate or low potency, particularly liver oils from dogfish and fish body oils. Molecular distillation can effect a five to fifty times concentration of this vitamin from oils of salt water fish, in which it occurs partly as a free alcohol and partly in the form of its esters *(38)*. Another method of concentration is that of cold fractionation of the oil using a special grade of propane as solvent, the vitamin A being concentrated in one of the fractions so obtained *(54)*. Solvents other than propane have been used for this purpose, and a similar technique has been suggested as a method for separating saturated from unsaturated glycerids in fish oils *(30)*. Vitamin A_2, which probably has a biological value equal to that of vitamin A, occurs in livers and viscera of fresh water fish. Recently a crystalline isomer of vitamin A has been isolated from the high potency liver oils of soupfin shark and halibut, and also from cod livers. This vitamin has been named Neovitamin A, and apparently accounts for 35 percent of the vitamin A in these fish liver oils *(84)*. Of interest is the fact that in young fish vitamin A potency of the oils is low and that it rises very markedly as the age of the fish increases *(83, 96)*. Much remains to be learned regarding the mechanism by which vitamin A is stored in fish livers. Vitamin A is quite unstable in most fish oils, though it appears to be protected in liver oils by natural antioxidants. In this connection it is of interest that very recent work has shown that 0.1 percent of α, β or γ tocopherols, especially when used in combination with 1 percent lecithin, considerably delays oxidative destruction of vitamin A in fish oils *(22)*. The destruction of vitamin A in dry powdered livestock feeds, where it is particularly liable to oxidation, is of economic importance, and methods of stabilizing it are being sought. There is always a danger that fish oils and consequently natural vitamin A will not be as plentiful as they have been, and signs of this are even now in evidence in the rapid depletion of the west coast soupfin shark, dogfish, and possibly pilchard or sardine fisheries. It may be that the time will come when some reliance must be placed in a synthetic source of vitamin A. The task of producing vitamin A in the laboratory is apparently an extremely difficult one. Some success has been achieved, and small amounts of biologically active related compounds have been obtained, though not in commercial quantities *(37, 53, 69)*.

Marine sources of the D vitamins continue to be exploited even with the advent of biologically active substances obtained by irradiation, or similar activation, of related sterols or "provitamins." The reason for this is largely because no provitamin of this type has been prepared by total synthesis, and partly because certain high potency vitamin D oils such as those of halibut and the percomorph fishes can be used directly as clinical sources of these vitamins. Vitamin D occurs largely as vitamin D_2 (viosterol and calciferol) and vitamin D_3 in fish liver oils, though there appear to be at least four other forms of this vitamin present *(38)*. The vitamin occurs partly in the free and partly in the esterified form. Man can utilize both vitamin D_2 and D_3, and fish liver oils are therefore a good source of these vitamins for humans. Vitamin D_2, which can be obtained by irradiation of yeast ergosterol, is apparently not utilized by young chicks, while vitamin D_3 is apparently one hundred percent active in this respect *(14)*. A natural source for a D provitamin for chicks was sought, and it was found that certain marine invertebrates, particularly mussels, yielded a provitamin of this type *(16, 17)*. Commercial use is being made of this discovery. Other marine sources of D provitamins are being sought. Thus one west coast firm is apparently extracting cholesterol, which sells for about $22.00 a pound, from fish meal by a special process which is estimated to cost about $17.00 per ton of treated meal. The yield of cholesterol is said to vary between 2 and

7 pounds per ton of meal. Cholesterol is also present in considerable quantities in certain fish body oils from which it might be prepared. 7-dehydrocholesterol can be obtained from cholesterol, and this in turn irradiated to give vitamin D_3. It has also been found that certain fish liver oils contain a form of vitamin D which is apparently much more active for chickens than vitamin D_3 *(14)*. Some attention is being given to the stabilization of vitamin D in poultry feeds *(32, 36)*.

An interesting development in the oil technology field is that of the use by the Norwegians of certain domestic fish body oils instead of imported olive oil in their canned sardines. The processes of manufacture of such oils apparently involve polymerization, but have remained secret, although the chemical and physical characteristics of the oils have been described *(41, 42, 43, 71, 72)*. Certain fish oils have considerable value for use as fine instrument lubricants. Thus porpoise, jackfish and blackfish jaw oils have long been employed for this purpose *(78)*. Ratfish liver oil *(86)* and Pacific porpoise oils *(95)* should also possess good lubricating properties. Ratfish liver oil mixed with pine oil has been said to have been used for massaging.

Phospholipids, probably lecithin, occur in salmon eggs. They can be obtained from the dried powdered material by first extracting with pentane to remove oil and pigments, and subsequently with a polar solvent such as methanol. Sockeye salmon roe yielded 12.5 percent oil and 6.2 percent phospholipids *(35)*.

MISCELLANEOUS

Numerous miscellaneous marine products have been described, and some of these may have commercial possibilities. Thus bile acids are found in fish, and some idea of the cost of producing these from salmon bile has been given *(26)*. Chitin, a polymerized acetyl glucosamine, is present in 16 to 22 percent concentration in shells of crustacea such as crabs, lobsters and shrimps. Glucosamine can be prepared from chitin by hydrolysis. It has also been suggested that chitin might form a useful alternative to cellulose for making adhesives, varnishes, wrapping materials, sausage casings etc. *(3)*. A number of sterols occur in sponges *(10, 101)* and in marine invertebrates *(9)*. Cholesterol has been found in the sea urchin, and sea cucumbers contain 7-dehydrocholesterol. Different corals have been found to contain cetyl palmitate *(58)*, cholesterol, hydrocarbons etc., and it is thought that coral reefs may play some role in petroleum formation *(8)*.

A rather profitable side line which has been developed on the west coast is that of production of sport fishing baits. Most of the methods used are kept secret, though publications concerning the preservation of salmon eggs *(23, 44)* and small whole fish *(97)* for bait are available. The preservative agents commonly employed in these baits include heat, formaldehyde, alum, borate, benzoates and salt.

Mention must be made before closing of the importance of seaweeds as sources of fishery by-products. It has been known for a long time that seaweeds are good sources of potash and iodine, and commercial use of this knowledge has been made. However, the most interesting products are the polysaccharide-like substances agar, alginic acid and the gelose (carragenin) of Irish moss. Several reviews concerning production methods and properties of these phyco-colloids are available *(67, 87, 88, 90, 98, 99, 104)*. Agar has abundant uses which include microbiology, pharmacy, medicine, dentistry, photography and food manufacture. It occurs in red seaweeds such as those known as *Gelidium* and *Gracilaria*. Alginic acid, which occurs in brown seaweeds such as kelp, and the carragenin of Irish moss, have valuable properties as stabilizers for ice cream, chocolate milk etc. A method for determining the stabilizing power of Irish moss extracts has been published recently

(81). Another development in the seaweed field is that of the production of rayon-like fibers from certain metallic alginates *(94)*. It is quite possible that other types of artificial fibers could be made from fish since filaments have been produced from collagen *(40)* and cat-gut like fibers from blood proteins *(25)*.

This review has necessarily been very brief, and it is probably inevitable that much interesting information has been omitted. Undoubtedly several large commercial concerns are carrying on research on fishery by-products, but the results of their work are not often made public. In closing I can only say that I hope that this talk has been of value to you, and that it may stimulate interest and investigation in this subject.

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Current Patents and Patent Litigation

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A MARVELOUS PRODUCT—BUT NOT PATENTABLE

The Supreme Court of the United States has just decided an extremely interesting and important patent case, *Funk Brothers Seed Company* v. *Kalo Inoculant Company*.

The action arose out of the Funk Company's alleged infringement of the product claims of Bond's Patent No. 2,200,532 owned by Kalo. The district court held the patent invalid; the Circuit Court of Appeals held it valid; and the Supreme Court consented to review the case.

An illustrative claim is as follows:

> "An inoculant for leguminous plants comprising a plurality of selected mutually non-inhibitive strains of different species of bacteria of the genus *Rhizobium*, said strains being unaffected by each other in respect to their ability to fix nitrogen in the leguminous plant for which they are specific."

Prior to Bond's invention it had been the practice to manufacture and sell inoculants containing only one species of root-nodule bacteria. The inoculant could only be used successfully in plants of the particular cross-inoculation group corresponding to the species. The six well-recognized species of bacteria and the corresponding cross-inoculation groups of leguminous plants are:

Rhizobium trifolii	Red clover, crimson clover, mammoth clover, alsike clover
Rhizobium meliloti	Alfalfa, white or yellow sweet clovers
Rhizobium phaseoli	Garden beans
Rhizobium leguminosarum	Garden peas and vetch
Rhizobium lupini	Lupines
Rhizobium japonicum	Soy beans

As a consequence, if a farmer had crops of clover, alfalfa and soy beans, it was necessary for him to use three separate inoculants. Although there had been a few mixed cultures for field legumes, they had proved generally unsatisfactory because the different species of the rhizobia bacteria produced an inhibitory effect on each other when they were mixed in a common base, so that their efficiency was reduced. As a result of the prior experience it had been assumed that the different species of rhizobia were mutually inhibitive.

Bond discovered that there are strains of each species of root-nodule bacteria which do not exert a mutually inhibitive effect on each other. He also ascertained that those mutually non-inhibitive strains, by certain methods of selection and testing, can be isolated and used in mixed cultures. As a result of his discovery he provided the art with a mixed culture of rhizobia which was capable of inoculating the seeds of plants belonging to several cross-inoculation groups. Thus there was made available to the farmer a single product which could be used for the inoculation of different leguminous plants.

The Supreme Court held that the product claims of the Bond patent were invalid. In arriving at its decision the Court stated that it was not considering the question

as to whether the methods of selecting and testing the non-inhibitive strains were patentable. The Court said:

> "Bond does not create a state of inhibition or of non-inhibition in the bacteria. Their qualities are the work of nature. Those qualities are of course not patentable."

It then proceeded to point out why such phenomena are not patentable:

> "The qualities of these bacteria, like the heat of the sun, electricity, or the qualities of metals, are part of the storehouse of knowledge of all men. They are manifestations of laws of nature, free to all men and reserved exclusively to none. He who discovers a hitherto unknown phenomenon of nature has no claim to a monopoly of it which the law recognizes."

The Court then summarized the basic principle which had long ago been enunciated and reiterated in famous cases:

> "If there is to be invention from such a discovery, it must come from the application of the law of nature to a new and useful end."

What the Court there said was that although electricity, for example, may not be monopolized for the limited period allowable under the patent statutes by an individual, a particular form of machine or instrument involving the use of electrical phenomena can be patented.

In bowling over the product claim of the Bond patent, the Court held that his product was an:

> "aggregation of species [which] fell short of invention within the meaning of the patent statutes."

The Court then proceeded to explain why it arrived at the foregoing conclusion.

> "Discovery of the fact that certain strains of each species of these bacteria can be mixed without harmful effect to the properties of either is a discovery of their qualities of non-inhibition. It is no more than the discovery of some of the handiwork of nature and hence is not patentable.
>
> "The aggregation of select strains of the several species into one product is an application of that newly discovered natural principle. But however ingenious the discovery of that natural principle may have been, the application of it is hardly more than an advance in the packaging of the inoculants. Each of the species of root-nodule bacteria contained in the package infects the same group of leguminous plants which it always infected. No species acquires a different use. The combination of species produces no new bacteria, no change in the six species of bacteria, and no enlargement of the range of their utility. Each species has the same effect it always had. The bacteria perform in their natural way. Their use in combination does not improve in any way their natural functioning. They serve the ends nature originally provided and act quite independently of any effort of the patentee."

It will be obvious that the kernel of the foregoing is the Court's statement that

> "the application of it [the discovery of a group of compatible bacteria] is hardly more than an advance in the packaging of the inoculants."

In other words, that it was obvious for Bond, on his discovery of the several strains of compatible bacteria, to put them into a single package.

The Court realized that a package of such a mixture is a valuable contribution. It said:

> "There is, of course, an advantage in the combination. The farmer need not buy six different packages for six different crops. He can buy one package and use it for any or all of his crops of leguminous plants. And, . . .

the packages of mixed inoculants also hold advantages for the dealers and manufacturers by reducing inventory problems and the like."

But a product must be more than new and useful to be patented; it must also satisfy the requirements of invention or discovery.

The Court then stated:

> "The application of this newly-discovered natural principle [the existence of compatible strains of bacteria] to the problem of packaging of inoculants may well have been an important commercial advance. But once nature's secret of the non-inhibitive quality of certain strains of the species of Rhizobium was discovered, the state of the art made the production of a mixed inoculant a simple step. Even though it may have been the product of skill, it certainly was not the product of invention. There is no way in which we could call it such unless we borrowed invention from the discovery of the natural principle itself. That is to say, there is no invention here unless the discovery that certain strains of the several species of these bacteria are non-inhibitive and may thus be safely mixed is invention. But we cannot so hold without allowing a patent to issue on one of the ancient secrets of nature now disclosed. All that remains, therefore, are advantages of the mixed inoculants themselves. They are not enough."

Mr. Justice Frankfurter wrote a separate opinion concurring in the conclusion of the Court, that the product claims were invalid, but based on altogether different reasons. He reviewed the prior state of the art and said that Bond had concluded that there might be special strains of the bacteria which lacked mutual inhibition, or were at all events mutually compatible. He pointed out that by using techniques that had previously been developed to test efficiency in promoting nitrogen fixation of various bacterial strains, Bond tested such efficiency of various mixtures of strains, thus confirming his notion that some strains were mutually compatible by finding that mixtures of these compatible strains gave good nitrogen fixation in two or more different kinds of legumes, while other mixtures of certain other strains proved mutually incompatible.

Mr. Justice Frankfurter then said that if his analysis (the foregoing) of Bond's endeavor was correct, two different claims of originality are involved:

(1) the idea that there are compatible strains
(2) the experimental demonstration that there were in fact some compatible strains.

With respect to the patentable character of a mixture or combination of compatible strains he disagreed with the majority's view that the combination was lacking invention, saying:

> "In so far as . . . the packaging of a particular mixture of compatible strains is an invention and as such patentable, I agree [with the Court below], provided not only that a new and useful property results from their combination, but also that the particular strains are identifiable and adequately identified."

He then points out what Bond failed to do.

> "I do not find that Bond's combination of strains satisfies these requirements. The strains by which Bond secured compatibility are not identified and are identifiable only by their compatibility."

He also refers to the record as showing that Bond did not even establish infringement.

> "Bond makes no claim that Funk Brothers used the same combination of strains that he had found mutually compatible. He appears to claim that

since he was the originator of the idea that there might be mutually compatible strains and had practically demonstrated that some such strains exist, everyone else is forbidden to use a combination of strains whether they are or are not identical with the combinations that Bond selected and packaged together."

Mr. Justice Frankfurter indicates where, in his opinion, the Circuit Court of Appeals had gone off on a tangent. He says:

> "The Circuit Court of Appeals seems to me to have proceeded on the assumption that only 'a particular composite culture' was devised and patented by Bond and then applies it to 'any composite culture' arrived at by deletion of mutually inhibiting strains, but strains which may be quite different from Bond's composite culture.
>
> "The consequences of such a conclusion call for its rejection. Its acceptance would require, for instance in the field of alloys, that if one discovered a particular mixture of metals, which when alloyed had some particular desirable properties, he could patent not merely this particular mixture but the idea of alloying metals for this purpose, and thus exclude everyone else from contriving some other combination of metals which, when alloyed, had the same desirable properties. In patenting an alloy, I assume that both the qualities of the product and its specific composition would need to be specified. The strains that Bond put together in the product which he patented can be specified only by the properties of the mixture."

With respect to the Court's opinion as to the laws of nature and work of nature, Justice Frankfurter said:

> "It only confuses the issue, however, to introduce such terms as 'the work of nature' and the 'laws of nature.' For these are vague and malleable terms infected with ambiguity and equivocation. Everything that happens may be deemed 'the work of nature,' and any patentable composite exemplifies in its properties 'the laws of nature.' Arguments drawn from such terms for ascertaining patentability could fairly be employed to challenge almost every patent. On the other hand, the suggestion that 'if there is to be invention from such a discovery, it must come from the application of the law of nature to a new and useful end' may readily validate Bond's claim."

He disagreed with the majority that the combination of mutually compatible strains, if properly identified, did not constitute invention:

> "Nor can it be contended that there was no invention because the composite has no new properties other than its ingredients in isolation. Bond's mixture does in fact have the new property of multi-service applicability. Multi-purpose tools, multi-valent vaccines, vitamin complex composites, are examples of complexes whose sole new property is the conjunction of the properties of their components. Surely the Court does not mean unwittingly to pass on the patentability of such products by formulating criteria by which future issues of patentability may be prejudged. In finding Bond's patent invalid I have tried to avoid a formulation which, while it would in fact justify Bond's patent, would lay the basis for denying patentability to a large area within existing patent legislation."

Mr. Justice Burton wrote a dissenting opinion which was concurred in by Mr. Justice Jackson. They agreed that Bond's combination satisfied the statutory requirements of invention or discovery. They disagreed, however, with Mr. Justice Frankfurter that the patent was invalid for want of a clear, concise description of how the combinations were made and used.

Mr. Justice Burton said:

> "The completeness and character of the description must vary with the subject to be described. Machines lend themselves readily to descriptions in terms of mechanical principles and physical characteristics.
>
> "On the other hand, it may be that a combination of strains of bacterial species, which strains are distinguished from one another and recognized in practice solely by their observed effects, can be definable reasonably only in terms of those effects.
>
> "In the present case, the patentee has defined the combinations in terms of their mutually inhibiting and non-inhibiting effects upon their respective abilities to take free nitrogen from the air and place it in the soil.
>
> "There is no suggestion as to how it would be reasonably possible to describe the patented product more completely."

His conclusion was that:

> "The patent covers all composite cultures of bacterial strains of the species described which do not inhibit each other's ability to fix nitrogen."

The decision of the Court in this case emphasizes the difficulty of predicting what will be the final conclusion as to the validity of a patent for a development which is a truly great contribution to the progress of the useful arts and sciences.

Industrial and Legal Viewpoints

WHAT FOOD TECHNOLOGY HAS DONE FOR OUR BUSINESS*

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How the rule-of-thumb procedures have been supplanted by scientific quality control is recounted. This change has come about during the life-time of most members of our Institute. Improved quality and reduced spoilage are the results of the change. Revolutionary changes in processing procedures are predicted.

Agriculture and the food industry have existed since the beginning of our civilization. The preservation of foods by drying, salting, and the use of an organic acid, such as vinegar, go back into antiquity. We, therefore, find ourselves in an industry that is not an outgrowth of research or laboratory work. The early scientists interested in this field found it necessary to study a great mass of tradition and prejudice. This may be one of the reasons why the career of the scientist in the food industry has not been quite so spectacular as it has been in some of our newer developed industries. Unfortunately, most of the efforts of scientists in the food field before this century were devoted to the chemical preservation of foods and much of this work fell into disrepute with the coming of Dr. Wiley's crusade for pure foods. The public got the impression that chemists in food factories spent their time developing various forms of adulteration with artificial colors, simulated flavors, and the use of poisons to preserve the product. There was enough truth in this to place all of us in industry in the early part of the century under considerable handicap.

At the Heinz Company it was felt that mother could satisfactorily preserve foods in the kitchen without the need of a chemist and there was, therefore, no need for chemists in the plant if good, clean methods and home recipes were used. Mr. H. J. Heinz I, founder of the company, who had so strongly supported Dr. Wiley in his crusade, felt so deeply on this point that the fact that we had a few scientists employed was kept carefully away from him. We did have several such scientists principally because Mr. Howard Heinz, son of the founder and then a Vice President, and Mr. Sebastian Mueller, Vice President in charge of Manufacture, felt there was a place for the scientific approach and that it could be made useful in developing a business. Mr. Howard Heinz was a graduate of Yale where he attended Sheffield, and Mr. Mueller had had some chemical training in Germany.

It was in such an atmosphere that I found myself, a young bacteriologist just out of a pre-medical course in college, puzzled as to just how I could make myself useful in a business that was so large, complex, and obviously developing very rapidly without any help from me or my kind of folks. Every operation was secret and the man who possessed the secret guarded it jealously, as he felt it was his only assurance of holding a job and being important. It was only when they got into difficulties and needed help that the scientifically trained worker had a chance to get some insight as to just what went on. Since these men carried out the processes by "rule of thumb" and their knowledge was often fallacious, they were usually at a loss as to how to correct a condition at all out of the ordinary that might develop. Fantastic causes were assigned. The moon was considered as having much to do with the success of food processing, and in my early years when we experienced a serious outbreak of spoilage in canned Baked Beans during the month of April, it was

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seriously suggested that the beans were simply germinating because it was very natural for them to do so at that time of the year.

As the young scientists demonstrated their ability to study these abnormal occurrences and correct the condition, they gradually gained the confidence of the men responsible for processing and were able to get a better insight as to what it was all about. This was not easy, however, and took much time and patience, and it was necessary to gear the development to the needs as they arose.

The salting of pickles is a typical example of how a comparatively simple operation was made to seem very difficult and mysterious. It seemed that only men with a certain God-given knowledge could successfully salt cucumbers into pickles. They were men of some standing who wore top hats and cutaway coats and of whom the management stood somewhat in awe, realizing that if they should happen to leave the business for any reason it would probably be wrecked. There was no one else available to carry on the processing. They usually determined the salting process by the sense of taste, and I still have a recollection of one of these very fancy looking gentleman poking a finger into a large tank of pickles, tasting the brine, and directing the number of pounds of salt to be added. As the salting of pickles under normal conditions is not too difficult a job, these men experienced fairly good results, although they did encounter spoilage at times.

To the scientifically trained youngster, it seemed that a definite process could very easily be worked out, controlled by the use of a hydrometer, to measure strength of brines, etc., and this developed in the Heinz Company into an issue which almost rocked it to its foundations. The two or three gentlemen responsible for the salting operations were men with years of experience who had been with the company a long period of time and stood well with the management. They almost succeeded in entirely discrediting the suggested formulated salting of pickles, but the younger men in the management saw that it got a trial, and, as a result, within a few years the salting of pickles was being done by high school students who worked for us during the summer and simply followed a typewritten sheet of instructions with results that were uniformly better than those enjoyed by the experts who had preceded them. This experience was rather typical of others which took the mystery out of vinegar manufacture, spaghetti drying, jelly making, etc.

It is probably somewhat surprising to our younger members to realize that there was a time when one had to be an Italian opera singer, or a reasonable facsimile thereof, to successfully dry spaghetti. I can still see our very outstanding Italian gentleman in charge of our spaghetti department sticking his hand out the window to feel the air and to determine just how to adjust his process. Of course, there was some sense to his feeling the air, but a wet and dry bulb thermometer proved to be much more accurate and effective, and after such control instruments were installed, the drying of spaghetti quickly became a very simple operation thoroughly understood by all the employees of the department.

The result of this scientific approach to processing was the occurrence of less spoilage, and, as the scientist evolved these control methods, in order to give a uniform finished product that would be satisfactory, he found that the changing of the name of our department to the Quality Control Department, rather than the Laboratory as it had always been termed, was a most happy one. It not only took away the stigma of chemists in the food business, but it really signified our function and gave everyone in the organization, including the scientists in the department, a clearer concept of just what their responsibility was. Since that time, the department has grown more rapidly in size and importance. Over the years it has spread its influence into all phases of production and at the present time is responsible for all

recipes and processes and gives functional direction to the manufacturing staff to enable them to properly carry out their operations.

In the case of raw material, it is necessary to understand what variations are apt to occur under certain conditions and to establish the characteristics of greatest significance for the particular use to which it is to be put. The preparation of this raw material also requires some attention from the Quality Control Department. The cooking operations prior to packaging are particularly vital and offer many problems to the people in the Quality Control Department.

Containers are another interesting field whether they be metal containers, such as tin cans, or glass containers with metal lids and rubber rings. The war years have particularly enlivened this area with the development of electrolytic tinplate and various substitute lacquers and enamels.

The processing of the packaged product to insure its keeping is very naturally almost entirely the responsibility of the scientist in the Quality Control Department. This department is interested also in the storage of finished products as these storage conditions have much to do with the quality of the product as it reaches the consumer.

With such a broad field to cover, it takes a well balanced team made up of agronomists, bacteriologists, chemists, physicists, micro-analysts, engineers, and, in a business such as ours, trained chefs because after all food is cooked in a kettle and not in a beaker. Such a team must of necessity have a wide variety of talents and temperaments, and the organization of such a team is not an easy task. Men in this field of work are by nature individualists, and it takes leadership of the highest order to weld them into a good combat team to carry out the functions and discharge the responsibilities of a Quality Control Department as it is now developed in most industries. Not only must the various fields of science be represented on the team, but the character of the work must be stratified into roughly Research, Applied Research, and Control. We must have Research spelled with a capital "R" and research spelled with a small "r", and it has been our experience that naturally the three fields overlap and blend. Where there is multiple plant operation, the problem is further complicated and it is necessary to firmly set up standards and specifications if uniformity is to be achieved. Laboratory methods must be standardized, factory procedures must be standardized, specifications for raw materials and supplies must be developed, and a strong factory quality control staff at each point should operate right on the firing line to see that these standards and specifications are met hour by hour throughout the operating day.

I do not think the development at the Heinz Company has been much different from that which has occurred in many other companies in the food business. Briefly, what has Food Technology contributed?

1. It has taken the mystery out of food preservation.
2. It has insured the consuming public of food products that are wholesome and safe to consume.
3. It has raised the standard of Quality in processed foods and developed a better degree of uniformity in this Quality.
4. It has permitted the production of processed foods on a more economical basis because it has prevented much of the waste that formerly occurred, and has permitted streamlining processes so that they could be carried out more economically.
5. It has made an outstanding contribution to our standard of living, enabling us to preserve our surpluses for times of need and to really keep the world living in times such as we are now going through when, without the various